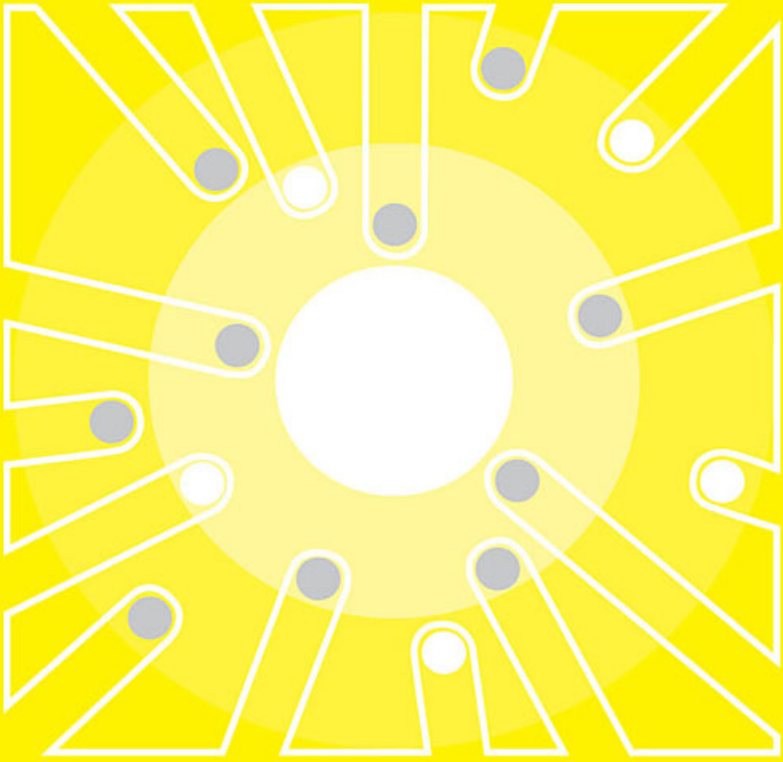
WHO FOOD ADDITIVES SERIES: 65

Safety evaluation of certain food additives and contaminants

Prepared by the
Seventy-fourth meeting of the Joint FAO/WHO
Expert Committee on Food Additives (JECFA)

Food and Agriculture Organization of the United Nations

World Health Organization



WHO FOOD ADDITIVES SERIES: 65

Safety evaluation of certain food additives and contaminants

Prepared by the Seventy-fourth meeting of the Joint FAO/ WHO Expert Committee on Food Additives (JECFA)

World Health Organization, Geneva, 2012

WHO Library Cataloguing-in-Publication Data

Safety evaluation of certain food additives and contaminants / prepared by the Seventy-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA).

(WHO food additives series ; 65)

1.Food additives - toxicity. 2.Food contamination. 3.Flavoring agents - analysis. 4.Flavoring agents - toxicity. 5.Risk assessment. I.Joint FAO/WHO Expert Committee on Food Additives. Meeting (74th : 2011 : Rome, Italy). II.World Health Organization. III.Series.

ISBN 978 92 4 166065 5 (NLM classification: WA 712)
ISSN 0300-0923

Typeset in India
Printed in Malta

CONTENTS

PREFACE

The monographs contained in this volume were prepared at the seventy-fourth meeting of the Joint Food and Agriculture Organization of the United Nations (FAO)/ World Health Organization (WHO) Expert Committee on Food Additives (JECFA), which met at FAO headquarters in Rome, Italy, on 14–23 June 2011. These monographs summarize the data on selected food additives and contaminants reviewed by the Committee.

The seventy-fourth report of JECFA has been published by the World Health Organization as WHO Technical Report No. 966. Reports and other documents resulting from previous meetings of JECFA are listed in Annex 1. The participants in the meeting are listed in Annex 3 of the present publication.

JECFA serves as a scientific advisory body to FAO, WHO, their Member States and the Codex Alimentarius Commission, primarily through the Codex Committee on Food Additives, the Codex Committee on Contaminants in Food and the Codex Committee on Residues of Veterinary Drugs in Foods, regarding the safety of food additives, residues of veterinary drugs, naturally occurring toxicants and contaminants in food. Committees accomplish this task by preparing reports of their meetings and publishing specifications or residue monographs and toxicological monographs, such as those contained in this volume, on substances that they have considered.

The monographs contained in this volume are based on working papers that were prepared by temporary advisers. A special acknowledgement is given at the beginning of each monograph to those who prepared these working papers. The monographs were edited by M. Sheffer, Ottawa, Canada.

The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the organizations participating in WHO concerning the legal status of any country, territory, city or area or its authorities, or concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the organizations in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological or toxicological properties of the compounds evaluated in this publication should be addressed to: Joint WHO Secretary of the Joint FAO/WHO Expert Committee on Food Additives, Department of Food Safety and Zoonoses, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland.

SPECIFIC FOOD ADDITIVES

ALUMINIUM-CONTAINING FOOD ADDITIVES (addendum)

First draft prepared by

D.J. Benford,[1] A. Agudo,[2] C. Baskaran,[1] M. DiNovi,[3] D. Folmer,[3] J.-C. Leblanc[4] and A.G. Renwick[5]

[1] Food Standards Agency, London, England
[2] Catalan Institute of Oncology, L'Hospitalet de Llobregat, Spain
[3] Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, Maryland, United States of America (USA)
[4] Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), Maisons-Alfort, France
[5] Emeritus Professor, University of Southampton, Southampton, England

1. EXPLANATION

Aluminium can occur in food as a result of its natural occurrence in the environment, contamination from various sources, leaching from food contact materials and the use of aluminium-containing food additives.

Various aluminium compounds were evaluated by the Committee at its thirteenth, twenty-first, twenty-sixth, twenty-ninth, thirtieth, thirty-third and sixty-seventh meetings (Annex 1, references *20*, *44*, *59*, *70*, *73*, *83* and *184*). At its thirteenth meeting, the Committee established an acceptable daily intake (ADI) "not specified" for sodium aluminosilicate and aluminium calcium silicate (Annex 1, reference *20*). At its twenty-sixth meeting, the Committee established a temporary ADI of 0–0.6 mg/kg body weight (bw) for sodium aluminium phosphate (Annex 1, reference *59*). At its thirtieth meeting, the Committee noted concerns about a lack of precise information on the aluminium content of the diet and a need for additional safety data. The Committee extended the temporary ADI of 0–0.6 mg/kg bw expressed as aluminium to all aluminium salts added to food and recommended that aluminium in all its forms should be reviewed at a future meeting (Annex 1, reference *73*).

The Committee evaluated aluminium as a contaminant at its thirty-third meeting, placing emphasis on estimates of consumer exposure, absorption and distribution of dietary aluminium and possible neurotoxicity, particularly

the relationship between exposure to aluminium and Alzheimer disease. The Committee established a provisional tolerable weekly intake (PTWI) of 0–7.0 mg/kg bw for aluminium, and a consolidated monograph was produced (Annex 1, reference *84*). The Committee concluded that there was no need to set a separate ADI for the food additives sodium aluminium phosphate basic or sodium aluminium phosphate acidic, because the PTWI included aluminium exposure arising from food additive uses.

At its sixty-seventh meeting, the Committee re-evaluated aluminium used in food additives and from other sources and concluded that aluminium compounds have the potential to affect the reproductive system and developing nervous system at doses lower than those used in establishing the previous PTWI (Annex 1, reference *186*). The Committee noted that the lowest lowest-observed-effect levels (LOELs) for aluminium in a range of different dietary studies in mice, rats and dogs were in the region of 50–75 mg/kg bw per day. The Committee selected the lower end of this range of LOELs (50 mg/kg bw per day) and established a PTWI of 1 mg/kg bw by applying an uncertainty factor of 100 to allow for interspecies and intraspecies differences and an additional uncertainty factor of 3 for deficiencies in the database, notably the absence of no-observed-effect levels (NOELs) in the majority of the studies evaluated and the absence of long-term studies on the relevant toxicological end-points. The PTWI applied to all aluminium compounds in food, including food additives. The previously established ADIs and PTWI for aluminium compounds were withdrawn. The Committee noted that the PTWI was likely to be exceeded to a large extent by some population groups, particularly children, who regularly consume foods that include aluminium-containing food additives. The Committee also noted that dietary exposure to aluminium is expected to be very high for infants fed on soya-based formula. The Committee noted a need for:

- further data on the bioavailability of different aluminium-containing food additives;
- an appropriate study of developmental toxicity and a multigeneration study incorporating neurobehavioural end-points using relevant aluminium compounds;
- studies to identify the forms of aluminium present in soya-based formula and their bioavailability.

Aluminium-containing food additives were re-evaluated by the Committee at its present meeting, as requested by the Codex Committee on Food Additives (CCFA). The Committee was asked to consider all data necessary for safety evaluation (bioavailability, developmental toxicity and multigeneration reproductive toxicity) and data on actual use levels in food. In addition, the Committee was asked to consider all data necessary for the assessment of safety, dietary exposure and specifications for aluminium lactate and potassium aluminium silicate, which had not been evaluated previously by the Committee for use as food additives. Potassium aluminium silicate is mined from natural sources and then further purified for use as a carrier substrate for potassium aluminium silicate–based pearlescent pigments. Potassium aluminium silicate–based pearlescent pigments are produced by reaction of potassium aluminium silicate with soluble salts of titanium and/or iron followed by calcination at high temperatures. The pigments can be produced with

a variety of different pearlescent colour effects depending upon particle size and the combination of titanium dioxide and/or iron oxide deposited on the potassium aluminium silicate.

The Committee received submissions from a number of sponsors, including unpublished studies of bioavailability and toxicity and a review of the scientific literature. Additional information was identified from the scientific literature. No information was received on the forms of aluminium present in soya-based infant formula.

Additional information was identified by searching PubMed for [aluminium and bioavailability] and [aluminium and neurotox*], focusing on studies likely to provide information on dose–response relationships.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

(a) Absorption

The bioavailability of a single dose of aluminium ammonium sulfate was assessed in groups of four male (302–379 g) and four female (236–265 g) fasted Crl:CD (SD) rats in a study that was compliant with good laboratory practice (GLP). Aluminium ammonium sulfate dissolved in physiological saline was administered by oral gavage at 300 and 1000 mg/kg bw and intravenously at 2 mg/kg bw. Blood samples were taken from the jugular vein at intervals up to 24 hours, and serum aluminium was measured by fluorescence detection liquid chromatography. Four of the top-dose animals (one male and three females) died and were replaced by additional animals. The cause of death in these animals is unclear. The bioavailability was calculated from the 24-hour area under the concentration versus time curve (AUC) values to be 0.039% in males and 0.061% in females dosed with aluminium ammonium sulfate at 300 mg/kg bw and 0.048% in males and 0.067% in females dosed at 1000 mg/kg bw (Sunaga, 2010a). If it is assumed that these doses were expressed as aluminium ammonium sulfate, the oral doses of aluminium would be 33 and 110 mg/kg bw, respectively.

The repeated-dose bioavailability of aluminium ammonium sulfate was assessed in groups of four male (267–293 g) and four female (183–198 g) Crl:CD (SD) rats in a study that was compliant with GLP. Aluminium ammonium sulfate dissolved in physiological saline was administered by oral gavage at 300 and 1000 mg/kg bw or intravenously at 2 mg/kg bw once daily for 14 days. Blood samples were taken from the jugular vein at intervals up to 24 hours after the final dosing, and serum aluminium was measured by fluorescence detection liquid chromatography. The bioavailability was calculated from the 24-hour AUC values to be 0.008% in males and 0.003% in females dosed with aluminium ammonium sulfate at 300 mg/kg bw and 0.006% in males and 0.023% in females dosed at 1000 mg/kg bw. The

maximum concentration (C_{max}) and AUC values increased in a dose-related manner between groups. There was no indication of accumulation. Comparison with the results of the single-dose study (Sunaga, 2010a) led the author to conclude that repeated administration resulted in decreased absorption of aluminium ammonium sulfate (Sunaga, 2010b). If it is assumed that these doses were expressed as aluminium ammonium sulfate, the oral doses of aluminium would be 33 and 110 mg/kg bw, respectively.

The bioavailability of a single dose of aluminium lactate was assessed in groups of four male (296–330 g) and four female (190–217 g) fasted Crl:CD (SD) rats in a study that was compliant with GLP. Aluminium lactate dissolved in physiological saline was administered by oral gavage at 300 and 1000 mg/kg bw or intravenously at 2 mg/kg bw. Blood samples were taken from the jugular vein at intervals up to 24 hours, and serum aluminium was measured by fluorescence detection liquid chromatography. The bioavailability was calculated from the 24-hour AUC values to be 0.067% in males and 0.164% in females dosed with aluminium lactate at 300 mg/kg bw and 0.161% in males and 0.175% in females dosed at 1000 mg/kg bw (Sunaga, 2010c). If it is assumed that these doses were expressed as aluminium lactate, the oral doses of aluminium would be 27 and 91 mg/kg bw, respectively.

The repeated-dose bioavailability of aluminium lactate was assessed in groups of four male (253–272 g) and four female (187–211 g) Crl:CD (SD) rats in a study that was compliant with GLP. Aluminium lactate dissolved in physiological saline was administered by oral gavage at 300 and 1000 mg/kg bw or intravenously at 2 mg/kg bw once daily for 14 days. Blood samples were taken from the jugular vein at intervals up to 24 hours after the final dosing, and serum aluminium was measured by fluorescence detection liquid chromatography. The bioavailability was calculated from the 24-hour AUC values to be 0.009% in males and 0.007% in females dosed with aluminium lactate at 300 mg/kg bw and 0.043% in males and 0.044% in females dosed at 1000 mg/kg bw. There was no indication of accumulation. Comparison with the results of the single-dose study (Sunaga, 2010c) led the author to conclude that repeated administration resulted in decreased absorption of aluminium lactate. The AUCs for the high-dose group were about 10–15 times greater than those for the low-dose group. The author considered the exceedance of the dose ratio to be due to disappearance of aluminium in blood at an early stage in the low-dose group and bimodal transition of serum aluminium concentrations in the high-dose group (Sunaga, 2010d). If it is assumed that these doses were expressed as aluminium lactate, the oral doses of aluminium would be 27 and 91 mg/kg bw, respectively.

The bioavailability of a single dose of aluminium sulfate was assessed in groups of four male (297–335 g) and four female (195–224 g) fasted Crl:CD (SD) rats in a study that was compliant with GLP. Aluminium sulfate dissolved in physiological saline was administered by oral gavage at 600, 1000 and 2000 mg/kg bw and intravenously at 1 mg/kg bw. Blood samples were taken from the jugular vein at intervals up to 24 hours, and serum aluminium was measured by fluorescence detection liquid chromatography. All of the top-dose animals, except for one female, died. The bioavailability was calculated from the 24-hour AUC values to be 0.046% in males and 0.064% in females dosed with aluminium sulfate at 600 mg/kg bw and

0.053% in males and 0.069% in females dosed at 1000 mg/kg bw (Sunaga, 2010e). If it is assumed that these doses were expressed as aluminium sulfate, the oral doses of aluminium would be 95 and 158 mg/kg bw, respectively.

The repeated-dose bioavailability of aluminium sulfate was assessed in groups of four male (247–270 g) and four female (184–213 g) Crl:CD (SD) rats in a study that was compliant with GLP. Aluminium sulfate dissolved in physiological saline was administered by oral gavage at 600, 1000 and 2000 mg/kg bw or intravenously at 1 mg/kg bw once daily for 14 days. Blood samples were taken from the jugular vein at intervals up to 24 hours after the final dosing, and serum aluminium was measured by fluorescence detection liquid chromatography. Dosing at 2000 mg/kg bw was discontinued as a result of deaths and loss of body weight. The bioavailability was calculated from the 24-hour AUC values to be 0.012% in males and 0.035% in females dosed with aluminium sulfate at 600 mg/kg bw and 0.012% in males and 0.052% in females dosed at 1000 mg/kg bw. The C_{max} and AUC values increased in a dose-related manner between groups. There was no indication of accumulation. Comparison with the results of the single-dose study (Sunaga, 2010e) led the author to conclude that repeated administration resulted in decreased absorption of aluminium sulfate in male rats. The C_{max} and AUC values increased in a dose-related manner between groups (Sunaga, 2010f). If it is assumed that these doses were expressed as aluminium sulfate, the oral doses of aluminium would be 95 and 158 mg/kg bw, respectively.

The oral bioavailability of aluminium compounds has been examined in studies using the long-lived radionuclide ^{26}Al as a tracer. Test animals were given food containing ^{26}Al orally, while a concurrent dose of ^{27}Al was given through intravenous infusion. The extent of oral absorption or bioavailability was determined by comparing the AUCs for aluminium given via the two routes. Compared with an experimental design in which a rat receives the two doses at different times, this method reduces variability by concurrently determining the AUCs from the oral and intravenous doses in the rat.

The bioavailability of acidic sodium aluminium phosphate incorporated into biscuits was examined in male Fischer 344 rats (322 ± 32 g, mean ± standard deviation [SD]). Biscuits were prepared with baking powder containing 25% sodium aluminium phosphate acidic, which is typical for baking powder. Five rats (which had been conditioned to eat biscuits containing sodium aluminium phosphate acidic) in each of two groups were given 1 g biscuit containing 1% or 2% sodium aluminium phosphate acidic that had ^{26}Al incorporated at known concentrations. The rats were concurrently intravenously infused with ^{27}Al at a dose of 100 µg/kg bw per hour (potassium aluminium sulfate was continuously infused from 14 hours prior to 60 hours after oral dosing) to produce an estimated aluminium concentration of 500 µg/l in the blood plasma to provide the ^{27}Al dose. Two control rats simultaneously received biscuits containing 1.5% sodium aluminium phosphate acidic, and one rat received an intragastric administration of 1 ml water, without ^{26}Al. Blood was withdrawn 1 hour prior to and up to 60 hours after oral dosing. The peak serum ^{26}Al concentration was increased by approximately 160-fold to 1840-fold above mean pretreatment values. Peak serum ^{26}Al concentrations in the 1% sodium aluminium phosphate acidic and 2% sodium aluminium phosphate acidic

groups occurred at 4.2 hours and 6 hours, respectively. The oral bioavailability was calculated to be 0.11% ± 0.11% and 0.13% ± 0.12% (mean ± SD), respectively, for the biscuits containing 1% and 2% sodium aluminium phosphate acidic, and the difference for the two groups was not statistically significant (Yokel & Florence, 2006). The authors reported that these results were significantly different from their previously reported bioavailability data for aluminium absorption from water (0.28% ± 0.18% for 1%; 0.29% ± 0.11% for 2%) using a similar method (Yokel et al., 2001; Zhou & Yokel, 2006).

In a similar experiment to determine the bioavailability of sodium aluminium phosphate basic in processed cheese, groups of six male Fischer 344 rats (272 ± 11 g, mean ± SD) were fed cheese containing 1.5% and 3% ^{26}Al incorporated into sodium aluminium phosphate basic. Three control animals received either processed cheese containing 2.5% sodium aluminium phosphate basic or intragastric administration of 1 ml of water without ^{26}Al. One rat was intravenously infused with ^{27}Al at a dose of 100 µg/kg bw per hour from 14 hours prior to 60 hours after oral dosing to produce an estimated aluminium concentration of 500 µg/l in the blood plasma to provide the ^{27}Al dose. This dose is below the transferrin binding capacity for aluminium (~1350 µg/l) and therefore will ensure that the chemical species are the same (aluminium–transferrin) for both ^{27}Al and ^{26}Al. Blood was withdrawn 1 hour prior to and up to 60 hours after oral dosing. Peak serum ^{26}Al concentrations were at least 200-fold above pretreatment values and occurred at 8.0 and 8.6 hours in the 1.5% and 3% sodium aluminium phosphate basic groups, respectively. The oral bioavailability was calculated to be 0.10% ± 0.07% and 0.29% ± 0.18%, respectively, for 1.5% and 3% sodium aluminium phosphate basic (Yokel, Hicks & Florence, 2008).

The same laboratory investigated aluminium bioavailability from a tea infusion using tracer ^{26}Al. ^{26}Al citrate was injected into tea leaves, providing about 0.65 mg aluminium (similar to the inherent quantity in tea leaves), and an infusion was prepared that contained 50 Bq (71.3 ng) ^{26}Al per millilitre. A similar infusate was prepared with non-^{26}Al-containing aluminium citrate. The infusions were given intragastrically to male Fischer 344 rats (312 ± 5 g, mean ± SD), which also received a concurrent intravenous ^{27}Al infusion. The oral bioavailability, estimated from the AUC, was 0.37% ± 0.26%. Compared with previous results, this was similar to that from water (0.28%), but significantly greater than that from sodium aluminium phosphate acidic in biscuits (0.12%) (Yokel & Florence, 2008).

A study was conducted to compare the bioavailabilities of different ^{26}Al-labelled aluminium compounds in groups of six female Sprague-Dawley rats. As oral doses, the amounts of aluminium administered were 1.47 ng ^{26}Al:50 mg ^{27}Al as citrate, 1.24 ng ^{26}Al:50 mg ^{27}Al as chloride, 1.77 ng ^{26}Al:50 mg ^{27}Al as nitrate, 2.44 ng ^{26}Al:50 mg ^{27}Al as sulfate (as solutions), 12.2 ng ^{26}Al:17 mg ^{27}Al as hydroxide, 17.9 ng ^{26}Al:23 mg ^{27}Al as oxide, 0.46 ng ^{26}Al:10 mg ^{27}Al as sodium aluminium phosphate acidic, 0.31 ng ^{26}Al:10 mg ^{27}Al as sodium aluminium phosphate basic and 0.60 ng ^{26}Al:27 mg ^{27}Al as sodium aluminosilicate (as suspensions in 1% carboxymethyl cellulose); and 0.96 ng ^{26}Al in 414 mg FD&C Red 40 aluminium lake, 2.4 ng ^{26}Al:26 mg ^{27}Al powdered pot electrolyte and 1.4 ng ^{26}Al:6.9 mg ^{27}Al as aluminium metal (mixed with honey and placed on the back of the tongue). Bioavailability was

assessed by comparing the amount of ^{26}Al remaining in the carcass after 7 days with that remaining 7 days after intravenous injection of 0.19 ng ^{26}Al as citrate. The 7-day time span was to ensure that all ingested aluminium had been cleared from the gastrointestinal tract and that the initial rapid clearance phase had been exceeded. The absorbed fraction was less than 0.3% for each of the different compounds. For the soluble aluminium compounds, it ranged from 0.05% to 0.2% (aluminium nitrate, 0.045%; aluminium chloride, 0.054%; aluminium citrate, 0.078%; aluminium sulfate, 0.21%). The absorbed fractions of sodium aluminosilicate and FD&C Red 40 aluminium lake were similar (0.12% and 0.093%, respectively). Uptakes of the other insoluble compounds were slightly lower (powdered pot electrolyte, 0.042%; aluminium hydroxide, 0.025%; aluminium oxide, 0.018%). Uptake of sodium aluminium phosphate acidic, sodium aluminium phosphate basic and aluminium metal could not be fully quantified because it was below the limit of detection (LOD); however, based on 50% of the LOD, uptake was reported to be <0.024%, <0.015% and <0.015%, respectively. The author noted that these results were consistent with those of human volunteer studies (Priest, 2010).

In an investigation of whether citrate, maltolate and fluoride significantly influence oral aluminium bioavailability, male Fischer rats were given intragastrically 1 ml of solution containing 37 Bq ^{26}Al (65 nmol total aluminium) as the Al^{3+} ion or as complexes with [^{14}C]citrate, [^{14}C]maltolate or fluoride, with concurrent ^{27}Al intravenous infusion. The aluminium bioavailability was estimated to be 0.29% ± 0.11%, 0.61% ± 0.31%, 0.50% ± 0.25% and 0.35% ± 0.10% from the ion, citrate, maltolate and fluoride, respectively. These differences were not statistically significant (Zhou, Harris & Yokel, 2008).

The solubility of six pigments consisting of potassium aluminium silicate (mica) coated with iron(III) oxide (0–56%) and/or titanium dioxide (0–52%) was investigated in model systems using simulated gastric (pH 1.231) and intestinal (pH 6.714) fluids, as an indicator of bioavailability, in a study conducted in compliance with GLP. The pigments were Candurin® Red Lustre, Candurin® Apple, Candurin® Silver Fine, Candurin® Gold Lustre and Candurin® Fudge. Aluminium silicate, iron(II) silicate and iron oxide were used as reference substances. The test materials were incubated with the simulated fluids for 2 hours at 37 °C, then insoluble material was separated by filtering, and the dissolved aluminium and iron were measured by atomic absorption. In the simulated gastric fluid, the solubility of aluminium from aluminium silicate (control) was 0.11%; for the six pigments, it ranged from 0.041% to 0.3%. In the simulated intestinal fluid, the solubility of aluminium from aluminium silicate (control) was 0.016%; for the six pigments, it ranged from 0.000 37% to 0.005%. The solubility of iron ranged from 0.0001% to 0.11% in the gastric fluid and from 0.000 33% to 0.024% in the intestinal fluid (St Laurent, 2006). In a data submission, it is argued that particles with a mean size greater than or equal to 5 µm are not taken up by the Peyer's patches and that lipophilic particles are taken up to a greater extent than hydrophilic particles; therefore, it is highly unlikely that insoluble potassium aluminium silicate particles in the range of 5–150 µm with a hydrophilic surface are absorbed by the gastrointestinal tract (Merck, 2010).

A wide variability in absorption was observed in a study in human volunteers. Four healthy males were given a capsule containing 960 mg aluminium hydroxide

(333 mg aluminium) with a drink of citrate and citrus juice to enhance absorption. Peak aluminium concentrations in serum were 31, 49, 426 and 766 µg/l in the four individuals. In five patients with Alzheimer disease given a 3-fold higher amount of aluminium hydroxide, the peak concentrations were 56–1447 µg/l (Molloy et al., 2007). The reported results of this study are insufficient to allow bioavailability to be estimated.

(b) Distribution

Groups of eight pregnant Wistar rats were given daily oral doses of aluminium chloride (presumably by gavage) of 0 or 345 mg/kg bw (70 mg/kg bw per day expressed as aluminium) on gestational days (GDs) 0–16. Standard laboratory diet and drinking-water were provided ad libitum; the exposure to aluminium from these sources was not estimated. Significantly higher levels of aluminium were detected in the blood, brain and placenta of the mothers and in the brains of fetuses compared with control animals. Additionally, groups of five lactating rats were given oral doses of aluminium chloride of 0 or 345 mg/kg bw (70 mg/kg bw per day expressed as aluminium) from days 0 to 16 postpartum. Following necropsy on day 20, higher levels of aluminium were detected in the brains of the pups of aluminium-treated animals, demonstrating transfer through the milk. Lesser increases in tissue aluminium levels were observed following co-administration of the chelator Tiron (disodium salt of 4,5-dihydroxy-1,3-benzene disulfonic acid) at 471 mg/kg bw intraperitoneally and/or reduced glutathione (GSH) at 100 mg/kg bw every other day throughout the period of aluminium dosing (Sharma & Mishra, 2006).

The relative distribution of aluminium was investigated in a GLP-compliant study with repeated oral administration of aluminium citrate, sulfate, nitrate, chloride and hydroxide to Sprague-Dawley rats. The animals were maintained on low-aluminium feed (9 µg/kg) and water (2 µg/l) for 17 days prior to and during dosing. The aluminium salts were administered by gavage to groups of five male (142–203 g) and five female (127–172 g) rats at doses corresponding to 30 mg/kg bw per day, expressed as aluminium, for 7 or 14 days. Control animals received deionized water. Appearance, body weights and feed and water consumption were monitored during the study. Blood samples were taken on day 8 or 15, prior to autopsy, when samples of brain, liver, kidney, spinal cord, spleen and bone were collected for analysis of aluminium.

There were no overt signs of toxicity or differences in body weight or water consumption. Feed consumption was significantly higher in the females dosed with aluminium nitrate compared with controls, but did not differ for other treatment groups. The concentrations of aluminium in most tissues were lower after 14 days of dosing than after 7 days, indicating that the major impact on the levels was the aluminium exposure prior to introduction of the low-aluminium diet and drinking-water for the study and that dosing with the different aluminium salts had a minimal effect. Aluminium citrate showed some evidence of systemic exposure, with concentrations higher than those of controls in the kidney and bone of males after 7 days and in the kidney of females after 14 days. Spinal cord concentrations of aluminium were higher in every group (including controls) after 14 days compared with 7 days (Dziwenka & Semple, 2009).

(c) Excretion

No new data on excretion were identified. Studies reviewed previously by the Committee have shown that urine is the primary route of excretion of absorbed aluminium in experimental animals and in humans. Initial half-lives of 2–5 hours have been reported in rats, mice, rabbits and dogs after intravenous administration and less than 1 day in humans. Multiple half-lives have been reported in different studies and species for a later, slower phase of elimination, varying with the tissue and generally increasing with the duration of sampling. EFSA (2008) concluded that although retention times for aluminium appear to be longer in humans than in rodents, there is little information allowing for extrapolation from rodents to humans.

2.1.2 Effects on enzymes and other parameters

Aluminium levels and enzymatic stress markers have been assessed in amyloid beta peptide transgenic mice, an animal model of Alzheimer disease, after oral aluminium exposure for 6 months. Amyloid beta peptide transgenic (Tg2576) and C57BL6/SJL wild-type mice 5 months of age were fed a diet containing aluminium lactate. The nominal aluminium concentration was 1000 mg/kg feed, but the actual level was 370 mg/kg feed, equal to 3.41 and 54 mg/kg bw per day in the control and treated mice, respectively. Aluminium levels were determined in the hippocampus, cerebellum and cortex, in addition to a suite of oxidative stress markers (GSH, oxidized glutathione, copper–zinc superoxide dismutase [SOD], glutathione reductase, glutathione peroxidase, catalase and thiobarbituric acid reactive substances [TBARS]), with and without co-exposure of the animals to deferoxamine. The highest levels of aluminium were observed in the hippocampus of both wild-type and transgenic mice. SOD activity was significantly decreased in the hippocampus of the aluminium-treated wild-type mice compared with the control wild-type mice. Glutathione reductase activity was significantly increased in the cortex of aluminium-treated wild-type mice compared with control wild-type mice (Esparza, Garcia & Gomez, 2011).

Groups of eight pregnant or five lactating Wistar rats were given daily oral doses of aluminium chloride (presumably by gavage) of 0 or 345 mg/kg bw (0 or 70 mg/kg bw per day, expressed as aluminium) on GDs 0–16 or on days 0–16 postpartum, respectively. Standard laboratory diet and drinking-water were provided ad libitum; the exposure to aluminium from these sources was not estimated (see also section 2.1.1(a)). Animals exposed to aluminium showed a number of indicators of oxidative stress in the brains of the mothers and in some instances also in the brains of the fetuses and sucklings. These were significant decreases in the levels of GSH, glutathione reductase, glutathione peroxidase, catalase, SOD and acetylcholinesterase and increases in the levels of TBARS and glutathione-*S*-transferase (GST). These effects were decreased by co-administration of the chelator Tiron (disodium salt of 4,5-dihydroxy-1,3-benzene disulfonic acid) at 471 mg/kg bw intraperitoneally and/or GSH at 100 mg/kg bw orally every other day throughout the period of dosing (Sharma & Mishra, 2006).

Groups of 10 male rabbits (1000–1100 g, strain not specified) were given aluminium chloride at 20 mg/l in drinking-water for 3 months alone or in combination with subcutaneous administration of melatonin, either for 15 days following or

simultaneously with the administration of aluminium chloride. A control group ($n = 5$) was included. The water intake was monitored weekly, and the aluminium chloride exposure was estimated at about 5–6.6 mg/day (approximately 1–1.3 mg/kg bw per day, expressed as aluminium). The aluminium contents of the diet and control tap water were not reported. After necropsy, the levels of malondialdehyde (MDA) and 4-hydroxyalkenal (4-HDA) (indicators of lipid peroxidation) and SOD activity were measured in the brain. The levels of MDA and 4-HDA were significantly increased, and SOD activity was decreased. These changes were lower in the groups treated with melatonin (as an antioxidant and free radical scavenger). The concentration of aluminium in the brain tissue was significantly increased in the aluminium-treated rabbits, and this change was also ameliorated by melatonin (Abd-Elghaffar, El Sokkary & Sharkawy, 2007).

Aluminium chloride was administered in the drinking-water for 6 months to male Wistar rats (young, 4 months; aged, 18 months; 10 animals in each treatment and control group), providing a dose of 50 mg/kg bw per day, expressed as aluminium. The aluminium content of the diet was not reported. When compared with controls, aluminium-treated rats showed a significant increase in electrophysiological activity. Histological examinations of hippocampal sections showed a decreased cell count in the CA1 and CA3 hippocampal fields with disorganized neurons that showed strong cytosolic staining in the aluminium-treated rats. The aluminium-treated rats showed oxidative stress–related damage to lipids (increased TBARS), decreased sodium–potassium adenosine triphosphatase (ATPase) activity, increased cytosolic protein kinase C activity and a significant decrease in the activity of SOD (Sethi et al., 2008). Curcumin administration by gavage (30 mg/kg bw per day) attenuated the changes (Sethi et al., 2009).

Aluminium chloride was administered in drinking-water to male Wistar rats (180–200 g; seven per group) at 100 mg/kg bw per day for 42 days. Additional groups of rats received concomitant doses of curcumin (30 and 60 mg/kg bw orally as a solution in 0.5% carboxymethyl cellulose 1 hour after aluminium chloride administration). No information was provided on levels of aluminium in food or control drinking-water. The animals were sacrificed on day 43, and biochemical indicators of oxidative stress were assessed in brain tissue. Aluminium chloride treatment resulted in a significant increase in brain levels of MDA and of nitrite (an indicator of nitric oxide production) and a decrease in GSH levels compared with controls. The treated rats also had a marked decrease in GST, SOD, catalase and acetylcholinesterase activities. The concentrations of aluminium were significantly increased in both the hippocampal and cortical areas of the brains of rats treated with aluminium chloride. Curcumin administration attenuated the changes in biochemical parameters and the increased aluminium concentration in the hippocampus, but not in the cortex (Kumar, Dogra & Prakash, 2009).

2.2 Toxicological studies

2.2.1 Acute toxicity

The Committee was provided with acute toxicity data on pigments consisting of potassium aluminium silicate (mica) coated with combinations of iron(III) oxide

Table 1. Acute oral toxicity of pigments consisting of potassium aluminium silicate (mica) coated with combinations of iron(III) oxide, titanium dioxide and myristic acid

Pigment	Species: number of each sex	Route	LD_{50} (mg/kg bw)	Reference
Iriodin® Ti 100K 68–76% mica 24–32% TiO_2	Rat: 5M + 5F	Oral gavage	>15 000	Von Eberstein & Rogulja (1970)
Iriodin® Color B Ti 100K 46–54% mica 46–54% TiO_2	Rat: 5M + 5F	Oral gavage	>15 000	Von Eberstein & Rogulja (1970)
Iriodin® Color Dy Ti 100K 51–65% mica 33–42% TiO_2 2–7% Fe_2O_3	Rat: 5M + 5F	Oral gavage	>15 000	Von Eberstein & Rogulja (1970)
Iriodin® Color G Ti 100K 46–50% mica 50–54% TiO_2	Rat: 5M + 5F	Oral gavage	>15 000	Von Eberstein & Rogulja (1970)
Iriodin® Color R Ti 100K 52–58% mica 42–48% TiO_2	Rat: 5M + 5F	Oral gavage	>15 000	Von Eberstein & Rogulja (1970)
Iriodin® Color Y Ti 100K 54–62% mica 38–46% TiO_2	Rat: 5M + 5F	Oral gavage	>15 000	Von Eberstein & Rogulja (1970)
Iriodin® Colibri Red-brown 47–57% mica ≤3% TiO_2 43–50% Fe_2O_3	Rat: 10M + 10F	Oral gavage	>16 000	Von Eberstein (1975)
Iriodin® 502 C 63 58% mica 40% TiO_2 2% myristic acid	Rat: 5M + 5F	Oral gavage	>5 000	Heusener & Von Eberstein (1988)[a]
Iriodin® Ti 100K 68–76% mica 24–32% TiO_2	Dog: 2M + 2F	Oral gavage	>6 400	Von Eberstein (1971)

F, female; LD_{50}, median lethal dose; M, male

[a] Conducted according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 401 in compliance with GLP.

(Fe_2O_3), titanium dioxide (TiO_2) and myristic acid. No lethality was reported at the maximum tested doses, corresponding to an aluminium concentration of greater than 2000 mg/kg bw (Table 1).

2.2.2 Short-term studies of toxicity

Two subchronic toxicity studies were conducted with potassium aluminium silicate (mica) coated with iron(III) oxide and/or titanium dioxide. According to the submission to the Committee, the formula of potassium aluminium silicate is $KAl_2[AlSi_3O_{10}](OH)_2$, which contains 20% aluminium based on atomic weights.

Iriodin® Ti 100K (69–75% mica, 25–31% titanium dioxide) was administered in the diet at a concentration of 0, 5000, 10 000 or 20 000 mg/kg to groups of 15 male (approximately 150 g) and 15 female (approximately 130 g) Wistar rats for 14 weeks, equivalent to 0, 500, 1000 and 2000 mg/kg bw per day as Iriodin® Ti 100K or approximately 0, 75, 150 and 300 mg/kg bw per day as aluminium. Five rats of each sex per group were followed up for a 10-week treatment-free recovery period. Clinical signs were checked daily, and feed consumption and body weights were recorded weekly during the study period. Blood and urine were sampled during and at the end of the study for haematological and biochemical analyses. At autopsy, the weights of 10 organs were recorded. Histopathological examinations were performed on a large range of organs of animals of all dose groups (five rats of each sex at 13 weeks, and three rats of each sex at the end of the recovery period). There were no treatment-related changes in any of the parameters recorded. Some histopathological findings were slightly increased in the treated animals, including fatty degeneration of the liver, hyperplasia of Kupffer cells and siderosis in the Kupffer cells and kidney. The authors considered that the siderosis could not be directly related to the test material, as the test material did not contain any iron, and haematological findings did not provide an indication that the siderosis could be related to phagocytosis of senescent or damaged erythrocytes. They concluded that there were no differences between the control and test groups and that the dietary concentration of Iriodin® Ti 100K of 20 000 mg/kg was the no-observed-adverse-effect level (NOAEL) (equivalent to about 300 mg/kg bw per day, expressed as aluminium) (Jochmann, 1972; Kramer & Broschard, 2000a).

Four potassium aluminium silicate–based pigments and a “placebo” (potash mica) were investigated in a subsequent study conducted according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 408, with the exception that ophthalmic examinations were not conducted. Potash mica (composition not provided, but assumed to be 100%), Iriodin® Ti 100 Color RY K (52.86% mica, 40.53% titanium dioxide, 6.6% iron(III) oxide), Iriodin® Colibri Red-brown K (52.84% mica, 1.87% titanium dioxide, 45.29% iron(III) oxide), Iriodin® Colibri Blue-green K (47.46% mica, 42.73% titanium dioxide, 9.81% chromium oxide) and Iriodin® Colibri Dark Blue (48.1% mica, 46.7% titanium dioxide, 5.2% Berlin blue) were administered in the diet at a concentration of 50 000 mg/kg to groups of 20 male (mean weight 183 g) and 20 female (mean weight 162 g) Wistar rats for 13 weeks. The control group consisted of 40 male and 40 female rats. Half of the animals from each group were maintained on untreated diet for a 2-month recovery period. These test material dietary concentrations were equal to doses of 3931 and 4370 (male and female), 3952 and 4466, 3983 and 4391, 3995 and 4418, and 3856 and 4362 mg/kg bw per day, respectively, equivalent to 786 and 875, 418 and 472, 421 and 464, 379 and 419, and 371 and 420 mg/kg bw per day, respectively, expressed as aluminium. Clinical signs were checked daily, and

feed consumption and body weights were recorded weekly during the study period. Blood and urine were sampled from half of the animals during and at the end of the study for haematological and biochemical analyses. At autopsy, organ weights were recorded. Histopathological examinations were performed on organs of all animals of all dose groups. Diarrhoea was reported in some treated animals during the 1st week, and soft faeces were observed occasionally throughout the treatment period, but not during the recovery period. Feed consumption was higher than that of controls in all treatment groups, including the potash mica group, as a result of the lower nutritional composition of the diets. Body weight gains did not differ between dose groups, except for some statistically significant slight increases at some time points in the females treated with Iriodin® Colibri Blue-green K and Iriodin® Colibri Dark Blue, which were not considered to be of biological significance. No other treatment-related effects were reported. The authors reported that no toxicological effects were observed in rats treated with pearlescent pigments at doses up to about 4000 mg/kg bw per day (Kieser, 1982; Kramer & Broschard, 2000b).

The findings of the above study were subsequently re-evaluated and confirmed (Hellmann & Broschard, 2005). The focus of this study was on the pigments containing approximately 50% potassium aluminium silicate carrier, and therefore the doses would correspond to about 400 mg/kg bw per day, expressed as aluminium. The “placebo” was potash mica without associated pigments, for which the dose expressed as aluminium was about 800 mg/kg bw per day.

2.2.3 Long-term studies of toxicity and carcinogenicity

Potassium aluminium silicate (mica) coated with titanium dioxide was tested in a combined oral chronic toxicity and carcinogenicity study in Fischer 344 rats, conducted in compliance with GLP. The test material was a 1:1 blend of two pigments with overall composition of 72% potassium aluminium silicate and 28% titanium dioxide and was administered at a concentration of 0, 10 000, 20 000 or 50 000 mg/kg in the diet to groups of 10 male and 10 female rats for 52 weeks and to groups of 50 male and 50 female rats for 130 weeks. At the start of the study, male rats weighed 104–166 g and female rats weighed 91–125 g. Body weights, feed consumption and gross signs of toxicity were recorded weekly during the first 14 weeks and then once every 4 weeks. Ophthalmic examinations were conducted before the study and then at weeks 52 and 104. Haematology, clinical chemistry, urinalysis, organ weights and gross and microscopic evaluations were reported.

There were no treatment-related findings in the 52-week study, except for test material coloration of the faeces in the top-dose group. In the carcinogenicity study, there were no differences in survival up to week 102, although survival of low-dose females was significantly lower than that of controls at termination. Mean body weights of high-dose males and mid- and high-dose females were significantly lower than those of controls at week 25, but not at the end of the study. The incidence of mononuclear cell leukaemia in male rats was 10/17, 10/16, 13/16 and 22/25, respectively, at 0, 10 000, 20 000 and 50 000 mg/kg diet, and the increased incidence in the high-dose group was ascribed to the greater survival compared with the other dose groups. There were no other treatment-related findings. The authors concluded that titanium dioxide–coated potassium aluminium silicate did

not produce toxicological or carcinogenic effects at dietary concentrations up to 50 000 mg/kg diet. This is equivalent to 2500 mg/kg bw per day of the test material or 360 mg/kg bw per day of aluminium (Pence & Osheroff, 1987; Bernard et al., 1990).

2.2.4 Genotoxicity

The Committee was provided with genotoxicity data on pigments composed of potassium aluminium silicate (mica) coated with combinations of iron(III) oxide and titanium dioxide. The preparations were negative for bacterial mutagenicity in the presence and absence of S9 and in an in vivo rat bone marrow micronucleus test (Table 2).

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

The reproductive toxicity of aluminium sulfate was investigated in a GLP-compliant study conducted according to OECD Test Guideline 416. Aluminium sulfate was dissolved in ion exchange water at 0, 120, 600 or 3000 mg/l. Groups of 24 male and 24 female Crl:CD (SD) rats (F_0 generation) were administered the aluminium sulfate from 5 weeks of age for 10 weeks prior to mating, during mating and gestation, when the parental males were culled, and, for the females, through weaning. Litters were normalized to eight pups on postnatal day (PND) 4. At weaning, 24 males and 24 females were selected to serve as the F_1 generation and were administered the aluminium sulfate for 10 weeks prior to mating, during mating and gestation, and, for the females, through weaning, as for the F_0 generation. The calculated mean exposures to aluminium sulfate during the treatment period were 8.6, 10.7, 14.4 and 15.3 mg/kg bw per day in the 120 mg/l group, 41.0, 50.2, 71.5 and 74.2 mg/kg bw per day in the 600 mg/l group and 188, 232, 316 and 338 mg/kg bw per day in the 3000 mg/l group, respectively, in F_0 males, F_1 males, F_0 females and F_1 females. The mean exposures to aluminium from the test substance were 1.36, 1.69, 2.27 and 2.41 mg/kg bw per day in the 120 mg/l group, 6.47, 7.92, 11.28 and 11.7 mg/kg bw per day in the 600 mg/l group and 29.7, 36.6, 49.8 and 53.3 mg/kg bw per day in the 3000 mg/l group, respectively, in F_0 males, F_1 males, F_0 females and F_1 females. The aluminium concentration of the unsupplemented water was less than 5 mg/l, and the aluminium content of the batches of diet used throughout the study was in the range of 25–29 mg/kg. The mean aluminium exposures from the diet were 1.6, 1.9, 2.2 and 2.3 mg/kg bw per day in the F_0 males, F_1 males, F_0 females and F_1 females of each group, respectively. The F_0 and F_1 parental generations were monitored for mortality, behaviour, body weights, feed and water consumption and reproductive performance. Spontaneous locomotor activity was assessed in 10 males and 10 females randomly selected from the F_1 generation at 4 weeks of age. Learning was assessed in a multiple T-maze in 10 males and 10 females randomly selected from the F_1 generation at 6 weeks of age. F_1 and F_2 pups were assessed for gross abnormalities, anogenital distance on PND 4 and developmental milestones. One male and one female pup per litter were tested for reflex responses on PNDs 5, 8 and 18. At autopsy, organs were weighed

Table 2. Genotoxicity of pigments composed of potassium aluminium silicate (mica) coated with combinations of iron(III) oxide and titanium dioxide

Test system	Test object	Test material	Dose	Results	Reference
In vitro					
Reverse mutation[a]	*Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537	Mica pigment mix (63.5% mica, 26.4% TiO_2, 10.1% Fe_2O_3)	5–5000 µg/plate	Negative	Utesch (2006)
Reverse mutation[a]	*Escherichia coli* WP2uvrA	Mica pigment mix (63.5% mica, 26.4% TiO_2, 10.1% Fe_2O_3)	5–5000 µg/plate	Negative	Utesch (2006)
In vivo					
Micronucleus formation[b]	Male Wistar rat bone marrow	Candurin® Honeygold (36–52% mica, 42–52% TiO_2, 6–12% Fe_2O_3)	2000 mg/kg bw, orally	Negative[c]	Utesch (2000)

S9, 9000 × *g* rat liver supernatant

[a] In the presence and absence of Aroclor-induced rat liver S9 mix.

[b] Killed at 24 and 48 hours.

[c] No change in the proportion of polychromatic erythrocytes.

and stored. Histopathological examination was conducted on reproductive organs of parental animals of the high-dose and control groups and on animals for which abnormal findings were recorded.

Water consumption was significantly decreased compared with controls in males and females of all treatment groups in a concentration-dependent manner. This was attributed to avoidance of the drinking-water because of the low pH (pH 3.57–4.20). In the 3000 mg/l groups, body weights, body weight gains and feed consumption were significantly decreased compared with controls in the F_0 males and females for up to 3 weeks after the start of administration, and feed consumption was significantly decreased in the F_1 generation. In the F_0 and F_1 females, there was a dose-related decrease in feed consumption during the 3rd week of lactation, which was statistically significant at 600 and 3000 mg/l. Deaths occurred in one F_1 male in each of the 120 and 3000 mg/l groups and in one F_0 female in the 600 mg/l group; these were not considered to be treatment related.

The females showed no significant effects of treatment on the estrous cycle, and there were no differences reported for copulation, fertility index, gestation index, precoital interval, gestation length, number of implantations, number of pups delivered or delivery index. There were no significant differences between groups for sperm parameters except for a significant decrease in absolute (but not relative) number of sperm in the 3000 mg/l F_0 males. In the F_1 and F_2 pups, there were no treatment-related differences in malformations, sex ratio or viability on PND 0, 4 or 21. At 3000 mg/l, the F_1 male and female pups had a significantly lower body weight on PND 21, and a similar, but not statistically significant, trend was seen in the F_2 pups. Body weights of F_1 and F_2 male and female pups at 3000 mg/l were significantly lower than those of controls at autopsy (PND 26). No significant differences were reported for age at completion of pinna unfolding, age at incisor eruptions, age at eye opening or anogenital distance in the F_1 and F_2 male pups or in the F_1 female pups. In the F_2 female pups, the completion time of pinna unfolding was significantly lower in the 600 mg/l group. The F_1 male pups showed no significant treatment-related differences in the time of preputial separation. In F_1 female pups, vaginal opening was significantly delayed in the 3000 mg/l group (mean ± SD: 31.4 ± 1.7 days vs 29.5 ± 2.1 days in control), although body weights at the time of vaginal opening were not significantly different.

No significant treatment-related differences were reported for righting reflex (PND 5), negative geotaxis reflex (PND 8) or mid-air righting reflex (PND 18) in the F_1 or F_2 pups, in locomotor activity assessed in F_1 males and females at 4 weeks or in the learning outcomes assessed in F_1 males and females at 4 and 6 weeks. There were no treatment-related macroscopic observations in the F_0 or F_1 parental generations at autopsy. In F_0 males, the absolute and relative liver weights and the absolute spleen weights were significantly decreased at 3000 mg/l relative to controls. In the F_1 males, the only statistically significant changes in organ weights were decreased absolute adrenal weight at 3000 mg/l and decreased absolute testis weight at 600 mg/l. There were no statistically significant differences in organ weights in F_0 or F_1 females. Histopathological examination revealed no treatment-related changes in the liver or spleen or in the reproductive organs.

In the F_1 and F_2 pups, absolute and, in some cases, also relative weights of liver and spleen were significantly lower at 3000 mg/l than in controls, but the organs showed no histopathological abnormalities. Absolute weights of thymus, kidneys, testes, epididymides, ovaries and uterus and relative thymus weights were also lower than those of controls, and relative brain weights were significantly higher in high-dose pups than in controls. These findings were considered to be secondary to the decreased body weights. Other findings were not dose related and were considered not to be treatment related. The authors concluded that, based on the retardation of sexual development in the F_1 females, attributed to inhibition of growth, and decreased body weight gain and liver and spleen weights in the F_1 and F_2 offspring, the NOAEL was 600 mg/l aluminium sulfate in the drinking-water, corresponding to 41.0 mg/kg bw per day (Fujii, 2009; Hirata-Koizumi et al., 2011a).

Expressed as aluminium, the reported NOAEL from this study equates to 6.47 mg/kg bw per day from the test substance plus at least 1.6 mg/kg bw per day from the diet—i.e. a total of about 8 mg/kg bw per day. The lowest-observed-adverse-effect level (LOAEL) from this study would be equivalent to a total of approximately 31 mg/kg bw per day, expressed as aluminium. However, in view of the clear treatment-related effects on fluid consumption and feed consumption of F_0 and F_1 dams during the 3rd week of lactation, it is not possible to ascertain whether the observations reported in the pups were a direct effect of the aluminium sulfate or due to decreased milk production by the dams, affecting pup weight on PNDs 21 and 26. In addition, grip strength was not measured, which limits comparison with the results of the studies used by the Committee in establishing the PTWI at its sixty-seventh meeting and with the study of Semple (2010) (see section 2.2.6).

The reproductive toxicity of aluminium ammonium sulfate was also investigated in a GLP-compliant study conducted according to OECD Test Guideline 416. Aluminium ammonium sulfate was dissolved in ion exchange water at 0, 50, 500 or 5000 mg/l. Groups of 24 male and 24 female Crl:CD (SD) rats (F_0 generation) were administered the aluminium ammonium sulfate from 5 weeks of age for 10 weeks prior to mating, during mating and gestation, when the parental males were culled, and, for the females, through weaning. Litters were normalized to eight pups on PND 4. At weaning, 24 males and 24 females were selected to serve as the F_1 generation and were administered the aluminium ammonium sulfate for 10 weeks prior to mating, during mating and gestation, and, for the females, through weaning, as for the F_0 generation. The calculated mean exposures to aluminium ammonium sulfate during the treatment period were 3.78, 4.59, 6.52 and 6.65 mg/kg bw per day in the 50 mg/l group, 33.5, 41.8, 58.6 and 61.9 mg/kg bw per day in the 500 mg/l group and 305, 372, 500 and 517 mg/kg bw per day in the 5000 mg/l group, respectively, in F_0 males, F_1 males, F_0 females and F_1 females. The mean exposures to aluminium from the test substance were 0.430, 0.522, 0.742 and 0.757 mg/kg bw per day in the 50 mg/l group, 3.81, 4.76, 6.67 and 7.04 mg/kg bw per day in the 500 mg/l group and 34.7, 42.3, 56.9 and 58.8 mg/kg bw per day in the 5000 mg/l group, respectively, in F_0 males, F_1 males, F_0 females and F_1 females. The aluminium concentration of the unsupplemented water was less than 5 mg/l, and the aluminium content of the batches of diet used throughout the study was in the range of 22–29 mg/kg. The mean aluminium exposures from the diet were 1.6,

1.8, 2.2 and 2.4 mg/kg bw per day in the F_0 males, F_1 males, F_0 females and F_1 females of each group, respectively.

The F_0 and F_1 parental generations were monitored for mortality, behaviour, body weights, feed and water consumption and reproductive performance. Spontaneous locomotor activity was assessed in 10 males and 10 females randomly selected from the F_1 generation at 4 weeks of age. Learning was assessed in a multiple T-maze in 10 males and 10 females randomly selected from the F_1 generation at 6 weeks of age. F_1 and F_2 pups were assessed for gross abnormalities, anogenital distance on PND 4 and developmental milestones. One male and one female pup per litter were tested for reflex responses on PNDs 5, 8 and 18. At autopsy, organs were weighed and stored. Histopathological examination was conducted on reproductive organs of parental animals of the high-dose and control groups and on animals for which abnormal findings were recorded.

Water consumption was decreased compared with controls in males and females of all treatment groups in a concentration-dependent manner. The decrease was statistically significant at 500 and 5000 mg/l in males and females of the F_0 and F_1 generations as well as at 50 mg/l in the F_0 males and at some times during treatment for the F_0 and F_1 females. These changes were attributed to avoidance of the drinking-water because of the low pH (pH 3.45–4.38).

There were no significant differences compared with controls in body weight, body weight gain or feed consumption in the 50 and 500 mg/l groups, except for a reduction in feed consumption in the F_0 females during the 1st week of treatment. At 5000 mg/l, body weight was decreased in the F_0 and F_1 males for up to 2 weeks after the start of administration; body weight gain and feed consumption were also decreased at this time in the F_0 males, but not in the F_1 males. In the females at 5000 mg/l, body weights were decreased during the first 1 or 2 weeks of treatment in both the F_0 and F_1 generations and after 3 weeks of lactation in the F_0 generation. Body weight gains were decreased during the first 1 or 2 weeks of treatment in both the F_0 and F_1 generations and after 3 weeks of lactation in the F_1 generation. Feed consumption was decreased in the F_0 females during the 1st week of treatment and during the 2nd and 3rd weeks of lactation in both F_0 and F_1 dams. One F_1 male in the 500 mg/l group died, which was not considered to be treatment related.

The females showed no significant effects of treatment on the estrous cycle, and there were no differences reported for copulation, fertility index, gestation index, precoital interval, gestation length, number of implantations, number of pups delivered or delivery index. There were no significant differences between groups for sperm parameters.

In the F_1 and F_2 pups, there were no treatment-related differences in malformations, sex ratio or viability on PND 0, 4 or 21. Decreased body weights were reported in the F_1 male and female pups at 5000 mg/l, but not in the lower dose groups. F_1 male pups had a significantly lower body weight on PND 21, F_1 female pups on PNDs 14 and 21, and F_2 male and female pups on the day of autopsy (PND 26). No significant differences were reported for age at completion of pinna unfolding, age at incisor eruptions, age at eye opening or anogenital distance in the F_1 and F_2 male and female pups. The F_1 male pups showed no significant

treatment-related differences in the time of preputial separation. In F_1 female pups, vaginal opening was significantly delayed in the 5000 mg/l group (mean ± SD: 32.3 ± 1.8 days vs 30.2 ± 2.1 days in control), although body weights were not significantly different at the time of vaginal opening.

No significant treatment-related differences were reported for righting reflex (PND 5), negative geotaxis reflex (PND 8) or mid-air righting reflex (PND 18) in the F_1 or F_2 pups, in locomotor activity assessed in F_1 males and females at 4 weeks or in the learning outcomes assessed in F_1 males and females at 4 and 6 weeks of age.

There were no treatment-related macroscopic observations in the F_0 or F_1 parental generations at autopsy. In the F_0 and F_1 males, there were no treatment-related changes in organ weights at 50 or 500 mg/l. At 5000 mg/l, absolute pituitary gland weight was significantly lower and relative kidney weight was significantly higher than those of controls in the F_1 males, but not the F_0 males. In the females, there was an apparent dose-related decrease in absolute pituitary weight in both the F_0 and F_1 generations, which was significantly different from control only at 5000 mg/l. Relative kidney weight was significantly increased at 500 and 5000 mg/l in the F_0 generation and at 5000 mg/l in the F_1 generation, but it did not show a dose-related trend. Absolute thymus weight in the high-dose F_1 females was significantly lower than that of control. There were no other statistically significant changes in organ weights. Histopathological examination revealed no treatment-related changes in the reproductive organs.

In the F_1 and F_2 male and female pups, there was an apparent dose-related decrease in absolute and relative thymus weights, which was significantly different from control at 500 and 5000 mg/l in the F_1 females, but only at 5000 mg/l in the other groups. Histological examination was not conducted on the thymus. Absolute and, in some cases, also relative weights of liver and spleen in the F_1 and F_2 pups were significantly lower at 5000 mg/l than in controls, but the organs showed no histopathological abnormalities. Absolute weights of kidneys, adrenals, testes and epididymides of the F_1 and F_2 male pups at 5000 mg/l were also lower than those of controls, whereas relative brain and kidney weights were significantly higher in high-dose pups than in controls. Changes in organ weights other than the thymus, liver and spleen were inconsistent in the female pups, with absolute weights of the adrenals and uterus significantly lower and relative weights of brain and kidney significantly higher in the F_1 pups and absolute weights of the ovary and uterus significantly lower and relative weights of brain, kidney and adrenals significantly higher in the F_2 pups. These findings were considered to be secondary to the decreased body weights. Other findings were not dose related and were considered not to be treatment related. The authors concluded that, based on the retardation of sexual development in the F_1 females, attributed to inhibition of growth, and decreased body weight gain and liver, spleen and thymus weights in the F_1 and F_2 offspring, the NOAEL was 500 mg/l aluminium ammonium sulfate in the drinking-water, corresponding to 33.5 mg/kg bw per day (Fujii, 2010; Hirata-Koizumi et al., 2011b).

Expressed as aluminium, the reported NOAEL from this study equates to 3.81 mg/kg bw per day from the test substance plus at least 1.6 mg/kg bw per day

from the diet—that is, a total of about 6 mg/kg bw per day. The LOAEL from this study would be equivalent to a total aluminium dose of approximately 35 mg/kg bw per day. However, in view of the clear treatment-related effects on fluid consumption and feed consumption of F_0 and F_1 dams during the later stages of lactation, it is not possible to ascertain whether the observations reported in the pups were a direct effect of the aluminium ammonium sulfate or due to decreased milk production by the dams, affecting pup weight on PNDs 21 and 26. In addition, grip strength was not measured, which limits comparison with the results of the studies used by the Committee in establishing the PTWI at its sixty-seventh meeting and with the study of Semple (2010) (see section 2.2.6).

(b) Developmental toxicity

The effects of oral exposure to aluminium and prenatal stress on the neurobehavioural performance of the offspring at 1 year (adult) and 2 years (old age) were studied in Sprague-Dawley rats. Aluminium exposure was in the form of aluminium nitrate in the drinking-water at concentrations providing aluminium doses of 50 (n = 15) and 100 (n = 21) mg/kg bw per day. Citric acid (355 and 710 mg/kg bw per day for the rats exposed to aluminium at doses of 50 and 100 mg/kg bw per day, respectively) was added to the drinking-water to increase the availability of aluminium. Basal aluminium levels in the diet and drinking-water were not reported. A subgroup in each category was subjected to restraint stress (2 hours per day on GDs 6–20) (n = 4 for aluminium exposure of 50 mg/kg bw per day and n = 5 for aluminium exposure of 100 mg/kg bw per day). Control animals (no exposure, no restraint, n = 17; restraint only, n = 11) were also used. The offspring continued to receive the aluminium exposure during lactation and the experimental period (1 or 2 years). Body weight and fluid intake were monitored weekly. Behavioural tests were conducted 1 and 2 years after birth. At the end of 1 and 2 years, there was no significant difference in the general motor activity (open-field test) between the controls and the exposed animals (with or without prenatal restraint). However, there was a difference in the spatial learning and retention tests (water maze test), with the animals of the lower-dose group (50 mg/kg bw per day, with or without restraint) performing better than animals of the high-dose group. Also, the 1-year-old rats (adult) performed better than the 2-year-old rats (old) in the water maze. Aluminium levels in the brains at the end of 2 years were elevated in the rats exposed to 100 mg/kg bw per day, but not 50 mg/kg bw per day. Animals that had the same exposure but were subject to prenatal restraint stress did not have high levels of aluminium in the brain (even though the behavioural parameters were not different), indicating that the prenatal stress prevents aluminium accumulation (Roig et al., 2006).

Groups of eight pregnant Wistar rats were given daily oral doses of aluminium chloride (presumably by gavage) of 0 or 345 mg/kg bw (70 mg/kg bw per day, expressed as aluminium) on GDs 0–16. Standard laboratory diet and drinking-water were provided ad libitum; the exposure to aluminium from these sources was not estimated. Body weights were monitored daily. The animals were necropsied on day 18, for the collection of uteri, maternal blood and brain and fetal brain. Body weight gain was significantly reduced in the aluminium-treated dams. The numbers

of corpora lutea and implantation sites, placental weight, crown–rump length and fetal weight were also reduced significantly. There were no gross or skeletal malformations in the fetuses, but there was a significant reduction in ossification of the parietal and caudal bones. These effects were, to some extent, ameliorated by co-administration of the chelator Tiron (disodium salt of 4,5-dihydroxy-1,3-benzene disulfonic acid) at 471 mg/kg bw intraperitoneally and/or GSH at 100 mg/kg bw every other day throughout the period of aluminium dosing (Sharma & Mishra, 2006).

The embryotoxic effects of aluminium chloride were studied in Sprague-Dawley rats. Groups of pregnant rats (240–250 g, n = 10) were given 0 or 50 mg aluminium chloride orally by gavage on GDs 1–3 (preimplantation) or GDs 4–6 (during implantation). This dose was approximately 200 mg/kg bw per day, expressed as aluminium chloride, or 40 mg/kg bw per day, expressed as aluminium. The exposure to aluminium from diet and drinking-water was not estimated. The animals were necropsied on day 20. The group exposed to aluminium chloride on GDs 1–3 showed a significantly increased number of resorptions (7.8% compared with 0% in the controls). Dosing on GDs 4–6 resulted in significantly reduced pregnancy rates and numbers of viable fetuses and significantly increased numbers of resorptions. Measures of maternal toxicity were not reported (Bataineh, Bataineh & Daradka, 2007).

A combined repeated-dose toxicity study on aluminium chloride basic with reproduction and developmental toxicity screening was conducted according to OECD Test Guideline 422, in compliance with GLP. Aluminium chloride basic consists of 17.0% aluminium oxide, 9.0% aluminium and 19.9% chlorine in aqueous solution. Groups of 10 male and 10 female Wistar rats were dosed by oral gavage with aqueous solutions of aluminium chloride basic at 0, 40, 200 and 1000 mg/kg bw per day (0, 3.6, 18 and 90 mg/kg bw per day, expressed as aluminium). Males were dosed for 28 days—that is, for 2 weeks prior to mating, during mating and up to termination. Females were dosed for 2 weeks prior to mating, during mating, through gestation and up to at least 3 days of lactation, comprising a total of 37–53 days. The aluminium content of the diet was not reported. The following observations were recorded: clinical signs, functional observations, body weights, feed consumption, haematological and clinical chemistry analyses, organ weights, and macroscopic and microscopic findings, with a particular focus on reproductive organs. Reproductive parameters included mating, fertility, conception, gestation duration, gestation index, percentage of live pups, postnatal loss, pup weights, sex ratio and clinical and behavioural signs during at least 4 days of lactation.

At the top dose, lower body weights and feed intake were reported in females during the first 3 weeks of the study, which the authors considered not to be of toxicological significance. At autopsy, there were signs of local irritation in the stomach in both sexes treated at the top dose, supported by histopathological observations of mild to moderate subacute inflammation of the glandular stomach. No other treatment-related changes were reported. The author considered that 1000 mg/kg bw per day was the NOAEL for systemic effects and 200 mg/kg bw per day was the NOAEL for local irritation (Beekhuijzen, 2007).

2.2.6 Special studies

(a) Neurotoxicity and neurobehavioural studies

Female transgenic (Tg2576) and wild-type mice were exposed for 6 months to aluminium lactate in the diet. The nominal concentration was 1000 mg/kg feed as aluminium, but the actual level was 370 mg/kg feed, equal to 3.41 and 54 mg/kg bw per day, expressed as aluminium, in the control and treated mice, respectively. General motor activity was evaluated using an open field, whereas spatial learning and memory were assessed in a water maze. No effects on general motor activity were found, whereas the open-field test showed an increased number of rearings in Tg2576 mice compared with the wild-type mice. Differences in learning were noted in the water maze acquisition test, in which aluminium-treated Tg2576 mice showed more difficulties in learning the task than aluminium-exposed wild-type mice (García et al., 2009).

The effect of chronic exposure to aluminium chloride on the function of the vestibulo-ocular reflex was examined in a study in which the vestibulo-ocular reflex was analysed to detect changes of the post-rotatory nystagmus main parameters with exposure to aluminium. Wistar rats (395–486 g) were subdivided into three groups (n = 90 each, 30 of which were used as controls, numbers of males and females unclear). Each group was further divided into animals of three different ages (3, 10 and 24 months). All the animals were given standard rat diet (mean aluminium levels 5.5 ± 0.1 µg/ml), and the water used to prepare the drinking solutions had aluminium levels of 40 ± 1 ng/ml. The control animals were given water with added sodium chloride (0.125, 0.25 and 0.5 g/l), whereas the exposed animals had aluminium chloride (0.5, 1 and 2 g/l) added to their drinking-water for 90 days (aluminium doses of 11.1 ± 2.5, 21.5 ± 4.2 and 43.1 ± 11.4 mg/kg bw per day, respectively, for the three groups). The vestibulo-ocular reflex was recorded every 10 days and at the end of the 90 days; the aluminium concentrations of the whole blood and (after necropsy) brain were measured in half of the animals, and immunohistochemistry studies were carried out on the remainder. There was no correlation between aluminium levels in the blood or in different compartments of the brain with animal age. However, there was a dose-related increase in the aluminium concentrations of the brainstem–cerebellum and the telencephalon. Post-rotatory nystagmus analysis was carried out with regard to onset latency, duration, frequency and amplitude of single jerks. Only animals with the highest aluminium exposure (43.1 ± 11.4 mg/kg bw per day) showed a significant impairment in all age groups, as shown by delayed onset latency, drastic reduction of its duration, jerk frequency and jerk amplitude. Immunohistochemical analysis of the brains of animals in the highest exposed group showed no difference in the number and shape of astrocytes and no amyloid deposits, regardless of age (Mameli et al., 2006).

In a pilot study, six male Wistar rats were fed a low-aluminium diet, providing 0.36 mg/kg bw per day twice weekly from age 5 months to age 16 months and then given aluminium chloride at 20 mg/l (as aluminium) in the drinking-water, providing a total aluminium dose of 1.52 mg/kg bw per day from food and water. From the age of 5 months, the rats were tested weekly in a T-maze task until the

end of their lifespan (averaging 29.8 months). Two of the six rats had significantly lower memory scores in “old age” compared with “middle age” (ages not defined) and exhibited “soft signs of dementia”, such as repetitive behaviour, indecision and inability to concentrate. Sections of brain were processed with the Walton bright field/fluorescent stain for aluminium; hippocampal neurons from the brains of all six rats showed varying extents of aluminium accumulation, with greater accumulation in the two rats showing memory deficits, whereas untreated rats aged 6 months were judged to be aluminium negative by this method. The author suggested that the two rats showing memory deficits absorbed more aluminium and were more susceptible to aluminium toxicity. However, there were no control animals in the study (Walton, 2007).

In a subsequent larger study, male Wistar rats were fed twice weekly on a restricted amount of low-aluminium diet from age 6 months to age 12 months, and then groups were given aluminium chloride at 0, 2 and 20 mg/l, expressed as aluminium, in the drinking-water, providing a total aluminium dose of approximately 0.4, 0.5 and 1.7 mg/kg bw per day from feed and water (group sizes of 13, 12 and 12, respectively). The dietary restriction was intended to reduce the rats’ weight to approximately 85% of the free-feeding weight, and typically the rats ate the feed in the first 2–3 days and had a day or more with no feed. The rats were tested weekly in a T-maze task until the end of their lifespan. In addition, gait characteristics were assessed once between 28 and 30 months. At necropsy, levels of γ-glutamyltranspeptidase, creatinine and aluminium were measured in serum, and brain sections were stained with a stain for aluminium developed by the author (modified Walton stain). Of the rats surviving to at least 28 months, 0/10 in the low-dose group, 2/10 in the intermediate-dose group and 7/10 in the high-dose group showed significantly lower performance in old age (>24 months) than in middle age (12–24 months). The rats with impaired performance had significantly higher serum aluminium levels and more aluminium in the entorhinal cortex cells of the brain. The author concluded that ingestion of aluminium at a dose of 0.5 mg/kg bw per day or more throughout most of adult life led, in old age, to a slowly progressing condition that impaired cognitive function in susceptible rats (Walton, 2009).

This study is difficult to interpret given the unusual feeding regime and the inconsistency with other studies that have not reported similar findings at much higher doses of aluminium. In addition, a submission received by the Committee noted 1) that the examiner was aware of the animals’ treatment group while assessing the cognitive outcomes, 2) that misclassification of exposure resulting from differences in individual animals’ feed and water consumption could not be excluded, 3) uncertainty regarding effects from frequent repeated administration of a neurobehavioural test in the same animals and 4) the lack of external validation of the method of staining for aluminium.

The effect of aluminium chloride on short- and long-term memory was examined in the offspring of lactating Wistar rats given aluminium chloride in their drinking-water at doses of 0, 200, 400, 600 and 800 mg/kg bw per day for 2 weeks. The pups were weaned at 37 days, and on day 45, they were trained in passive avoidance response, then tested for short- and long-term memory 2 and 30 days after the training. Two criteria were considered: latency in entering a dark chamber

(step-through latency) and the time spent in the dark chamber. Drinking-water consumption was not reported. There were no statistically significant differences from controls at aluminium chloride doses up to 600 mg/kg bw per day. Offspring of dams dosed at 800 mg/kg bw per day exhibited a decrease in step-through latency at both 2 days and 30 days after training, but not in the time spent in the dark chamber (Ali, Vostacolaee & Rahim, 2008).

Groups of 10 male rabbits (1000–1100 g, strain not specified) were given aluminium chloride at 20 mg/l in drinking-water for 3 months alone or in combination with subcutaneous administration of melatonin, either for 15 days following or simultaneously with the administration of aluminium chloride. A control group ($n = 5$) was included. The water intake was monitored weekly, and the aluminium chloride exposure was estimated at about 5–6.6 mg/day (approximately 1–1.3 mg/kg bw per day, expressed as aluminium). The aluminium contents of the diet and control tap water were not reported. After necropsy, the brains of the animals were subject to neuropathological examination. Atrophy and apoptosis of the neurons in the cerebral cortex and hippocampus, associated with neurofibrillary degeneration and argyrophilic inclusion, Schwann cell degeneration and nerve fibre demyelination, were reported in the aluminium-treated rabbits. These effects were lower in the groups treated with melatonin (as an antioxidant and free radical scavenger) (Abd-Elghaffar, El Sokkary & Sharkawy, 2007).

The effects of aluminium exposure on the glial system and behaviour of Wistar rats were examined in a study involving administration of aluminium chloride at 3 g/l in the drinking-water to adult (3-month-old) rats for 4 months or to female rats (n = 10) during gestation and lactation and then to their offspring until they were 4 months old. Two control groups (n = 5 each) of 7- and 4-month-old pups were also examined. No information was provided on the aluminium content of the food. Effects on the glial system were evaluated using immunohistochemistry for glial fibrillary acidic protein. Glial fibrillary acidic protein labelling and the numbers of astrocytes were increased in the brains of aluminium-treated rats compared with controls. Both groups of aluminium-treated rats showed significantly reduced locomotor activity compared with controls. The rats exposed in utero also exhibited significantly increased time in the lit compartment of a dark/light box (indicating increased anxiety), which was not seen in the rats exposed only as adults (Erazi, Sansar & Ahboucha, 2010).

Female Wistar rats (180 ± 4 g, n = 7) were given aluminium nitrate in drinking-water at a concentration of 0 or 80 mg/l for 90 days. No information was provided on the actual dose or the aluminium content of the food. Body weights were recorded weekly; motor activity in an open-field test and memory in a novel object recognition task were examined once every fortnight alternately. Brain aluminium concentration was evaluated at the end of the study. Body weights of treated rats were significantly lower than those of controls in weeks 12 and 13. There were no statistically significant differences in motor activity throughout the study. Treated rats exhibited a significant deficit in the recognition memory test in weeks 8 and 10 compared with the controls. There was no significant difference in the concentration of aluminium in the brain (Azzaoui, Ahami & Khadmaoui, 2008).

Aluminium chloride (100 mg/kg bw per day, expressed as aluminium) was given orally to rats for 6 weeks. On the 3rd week (21st day) and 6th week (42nd day) of the study, various behavioural tests (Morris water maze and elevated plus maze task paradigms) and locomotion (photoactometer) were conducted to evaluate cognitive performance. The rats were killed on the 43rd day following the last behavioural test. The aluminium treatment resulted in poor retention of memory in the Morris water maze and elevated plus maze task paradigms (Prakash & Kumar, 2009).

Aluminium toxicity and possible protection due to antioxidant effects of curcumin were studied in male Wistar rats (180–200 g; seven per group). Aluminium chloride was administered in drinking-water at 100 mg/kg bw per day for 42 days. Additional groups of rats received concomitant doses of curcumin (30 and 60 mg/kg bw orally as a solution in 0.5% carboxymethyl cellulose 1 hour after aluminium chloride administration). No information was provided on levels of aluminium in feed or control drinking-water. Behavioural studies were carried out on the 21st and 42nd days following training on day 20, to evaluate memory and locomotion. Aluminium chloride–treated rats showed a significant cognitive impairment in a spatial navigation task and significant memory impairment in an elevated plus maze task. No significant differences in locomotor activity between treated rats and controls were observed. Rats treated with curcumin showed improved cognitive performance and memory retention compared with those treated with aluminium chloride alone (Kumar, Dogra & Prakash, 2009).

The effects of aluminium on spatial learning and neurogenesis were studied in the transgenic mouse (Tg2576) model of Alzheimer disease. Groups (n = 7–8) of 5-month-old male Tg2576 mice and wild-type control mice were fed normal chow diet supplemented with aluminium lactate at 0 or 1000 mg/kg (0 or 101 mg/kg as aluminium, according to the authors) for 120 days. No information was provided on the content of aluminium in the chow or drinking-water. During the 4th month of treatment, activity in an open-field test and learning in a water maze were evaluated. The mice were then injected intraperitoneally with 5-bromo-2′-deoxyuridine at 100 mg/kg bw per day for 2 consecutive days and sacrificed 1 and 28 days after the last injection in order to study hippocampal cell proliferation and differentiation. In general, the aluminium-treated mice of both genotypes drank more water and ate less feed throughout the study. Although some differences were observed between the genotypes, this study did not demonstrate consistent effects due to the aluminium. The major observation was that in the Morris water maze, aluminium impaired learning and memory in the wild-type mice, but not in the transgenic mice. Aluminium treatment did not affect motor activity in either transgenic or wild-type mice (Ribes et al., 2008).

Aluminium chloride was administered in the drinking-water to male Wistar rats (young, 4 months old; aged, 18 months old; 10 animals in each treatment and control group) for 6 months, providing an aluminium dose of 50 mg/kg bw per day. The aluminium content of the diet was not reported. Cognitive outcomes were determined using the open-field test (locomotor activity: horizontal [ambulation] and vertical [rearing]), defecation index (number of faecal boluses) and the Morris water maze. The cognitive tests were administered at the end of exposure, just prior

to sacrifice. The open-field test showed a statistically significant detrimental effect of age on both horizontal and vertical activity, but no statistically significant effect of aluminium treatment on these parameters. Aluminium exposure was associated with an increase in faecal index in both age groups, with a larger increase in the young animals. Results of the Morris water maze were reported as mean latency to reach a hidden platform. On day 1, the young aluminium-treated rats required significantly longer than the young controls, whereas the aged aluminium-treated rats showed mean latencies similar to those of the aged controls. By day 4, the difference between the young aluminium-treated rats and young control rats had diminished. The difference between the aged aluminium-treated rats and aged control rats increased from day 1 to day 4, with the aluminium-treated rats showing a decreased ability to learn over the 4-day period (Sethi et al., 2008).

The developmental and chronic neurotoxicity of aluminium citrate was investigated in Sprague-Dawley rats in a study conducted according to GLP with a design based on OECD Test Guideline 426. Aluminium citrate was administered in drinking-water to groups of pregnant rats, commencing on GD 6, at concentrations aiming to deliver aluminium doses of 30, 100 and 300 mg/kg bw per day, based on an expected water intake of 120 ml/kg bw per day. Two control groups received either sodium citrate solution (27.2 g/l), the molar equivalent of the high-dose aluminium citrate, or plain water. The concentration of aluminium in the diets was 7–8.5 ng/ml, which would have contributed less than 1 µg/kg bw per day. After delivery, 20 litters per dose group were selected for the study, and the litters were culled to four males and four females. One male and one female per litter were assigned to one of four milestone groups designated for neurobehavioural testing on PNDs 23, 64, 120 and 364. Weaned pups received the same treatment as the dams. Actual doses were near or above target in the dams. Observations in the dams included water consumption, body weight, a functional observational battery, morbidity and mortality. Actual doses were one third to one half of the target doses in the pups for most of the 1-year treatment period owing to lower than expected fluid consumption. Observations on the pups included body weight twice weekly, fluid consumption weekly and a functional observational battery on all pups several times before weaning and twice weekly on the 1-year group until sacrifice. Motor activity, startle response and performance in a T-maze test and the Morris water maze test were assessed at various times. At each sacrifice time, half of the pups of each group were processed for neurohistopathological examination, and the other half were subjected to a regular necropsy followed by brain weight measurement, clinical chemistry, haematology, and collection of tissues and blood for measurement of aluminium and other metals.

There were no consistent effects of aluminium citrate on the dams, except for increased fluid consumption at the low and middle doses. The most notable treatment-related effect observed in the offspring was renal damage (hydronephrosis, urethral dilatation, obstruction and/or presence of calculi), most prominently in the male pups. Higher mortality and significant morbidity, apparently due to urinary tract pathology, were observed in the male pups in the high aluminium citrate dose group, leading to termination of this group on day 98. Thus, the high-dose group was likely to be close to the maximum tolerated dose. Effects seen at the middle dose included urinary tract lesions, lower body weight in the males at PND 120

compared with controls, elevated fluid consumption in males and females, and an exaggerated response to tail pinch and narrower foot splay in the females. Apart from the urinary tract pathology, the most consistent and dose-related effect was decreased hindlimb and forelimb grip strength in both male and female pups. No consistent treatment-related effects were observed in ambulatory counts (motor activity) in the different cohorts. No significant effects were observed in the tests for learning or memory. None of the lesions seen on histopathological examination of brain tissues of the day 364 group were reported as treatment related, and, as these were also seen in the control group, the lesions were likely due to ageing. Tissue levels of aluminium were generally dose related, with the level in the bone showing the strongest association. Levels in blood were higher than those in the tissues. Of the central nervous system tissues, the highest level was in the brainstem. Overall, the authors concluded that the study indicated a LOAEL of 100 mg/kg bw per day and a NOAEL of 30 mg/kg bw per day (Semple, 2010; Poirier et al., 2011).

Identification of the LOAEL and NOAEL in this study is complicated by the decreasing fluid consumption and uncertainty regarding the critical exposure period. In the low-dose group, the achieved dose was about 40 mg/kg bw per day in the 1st week post-weaning, decreasing to 30 mg/kg bw per day (target dose) by week 5, and was about 15–45% of the target dose from post-weaning week 13 onwards. In the mid-dose group, the achieved dose was about 190 mg/kg bw per day in the 1st week post-weaning, decreasing to 100 mg/kg bw per day (target dose) by week 7, and was about 25–50% of the target dose from post-weaning week 15 onwards.

2.3 Observations in humans

The last evaluation by the Committee (Annex 1, reference *186*) considered all data relevant to the toxicity of and exposure to aluminium used in food additives and from other sources. A number of epidemiological studies were reviewed, most of them focusing on the potential association of oral exposure to aluminium in water, food or antacids with Alzheimer disease and cognitive impairment. Some studies suggested an association between consumption of aluminium in water and Alzheimer disease, but such an association was not confirmed in other studies. Only one of these studies assessed the ingestion of bottled water, whereas the remaining studies relied on concentrations of aluminium in water supply as a measure of exposure. None of them accounted for the ingestion of aluminium in foods, a potentially important confounding factor, as the aluminium in drinking-water represents a minor source of oral exposure. There was minimal information about the potential neurotoxic effects of aluminium in food, and the studies of the use of antacids did not demonstrate an association with neurological conditions. There were also a few case reports of adults and a child with normal kidney function who experienced skeletal changes attributed to frequent use of aluminium-containing antacids, considered to induce phosphate depletion. In summary, no pivotal epidemiological studies were available for the risk assessment in the previous evaluation.

The European Food Safety Authority (EFSA) also provided a scientific opinion on the safety of aluminium from all dietary sources (EFSA, 2008). EFSA

evaluated the neurotoxicity in patients undergoing dialysis in which insufficiently purified water was used and there was parenteral exposure to high concentrations of aluminium, as well as the potential role of aluminium in the etiology of Alzheimer disease and its association with other neurodegenerative diseases. However, these hypotheses remain controversial. There were very few specific toxicological data for food additives containing aluminium, and the available studies had a number of limitations and did not allow any dose–response relationships to be established.

Since the last evaluation by the Committee (Annex 1, reference *186*), a few epidemiological studies have been published on the association between exposure to aluminium and Alzheimer disease, dementia and other neurological outcomes, mainly among subjects exposed through drinking-water, but also in people following ingestion of antacids, children exposed from parenteral nutrition and workers with potential occupational exposure. Some of these studies were already included in the EFSA evaluation (EFSA, 2008), as well as in the recent report by Risk Sciences International (RSI, 2010).

2.3.1 Biomarkers of exposure

No studies on biomarkers of aluminium exposure were found.

2.3.2 Biomarkers of effects

No studies on biomarkers of aluminium effects were found.

2.3.3 Clinical observations

(a) Case reports

A case of possible relevance was reported for a woman who had been acutely exposed to high aluminium concentrations in drinking-water as a result of an accidental discharge of aluminium sulfate into the local mains water supply in Cornwall, England. Fifteen years later, the woman, by then aged 58 years, was referred for investigation of deterioration of her mental state; she continued to deteriorate and died within 1 year, and an autopsy was performed. A rare form of sporadic early-onset β-amyloid angiopathy in cerebral cortical and leptomeningeal vessels and in leptomeningeal vessels over the cerebellum was identified. A few neurofibrillary tangles (NFT) were observed in the cortex and hippocampus. In addition, high concentrations of aluminium were found coincident with the severely affected regions of the cortex. According to the authors, a causative role for aluminium in the development of the observed neuropathology cannot be concluded, although the association between high brain aluminium levels and unusual neuropathology deserves further investigation (Exley & Esiri, 2006).

Another case was reported for a 20-year-old woman who fell into a coma with anisocoria and left spastic hemiparesis after respiratory infection; her condition was slowly progressive and developed into a vegetative state. Brain imaging showed massive abnormal signals in the white matter. Electron spectroscopic imaging of biopsied brain tissue confirmed that the electron-dense deposits were associated with aluminium accumulation in the myelin sheath. The probable source

of exposure to aluminium was unknown. Myelin is known to easily become a primary target of aluminium toxicity, because aluminium binds to transferrin and is taken into oligodendrocytes, and this may have contributed to aluminium-induced toxicity (Itoh et al., 2008).

(b) Aluminium in brain and Alzheimer disease

A few studies have reported the presence of aluminium in brain tissue, often associated with neuropathological features of Alzheimer disease. One study assessed the localization of aluminium in corticolimbic neurons of six patients with autopsy-confirmed Alzheimer disease and six non-demented controls. All pyramidal neurons in these specimens appeared to exhibit at least some degree of aluminium staining. On the basis of their staining patterns, all pyramidal neurons could be classified into two stages: progressive increase of nuclear aluminium (often accompanied by granulovacuolar degeneration with granules that stain for aluminium) or formation of NFT in regions of aluminium-rich cytoplasm, especially in brain tissue of patients with Alzheimer disease. Given that the NFT in human neurons always developed in conjunction with cytoplasmic aluminium, it was hypothesized that aluminium may play a role in their formation (Walton, 2006). In a subsequent study, hippocampal cells from the brains of five patients with confirmed Alzheimer disease and five non-demented controls were examined. Mature NFT were observed in all the Alzheimer disease cases and three of the controls. NFT stained for both aluminium and hyperphosphorylated tau. Overall, the results showed co-localization of aluminium and hyperphosphorylated tau in an Alzheimer disease–vulnerable region of the brain (Walton, 2010).

In another study, the brains of patients with Alzheimer disease were examined using transmission electron microscopy energy-dispersive X-ray spectroscopy. The results from this study demonstrated the presence of aluminium in amyloid fibres in the cores of senile plaques located both in the hippocampus and in the temporal lobe (Yumoto et al., 2009).

Amyloid plaques and NFT are prominent neuropathological hallmarks of Alzheimer disease. Both the accumulation of aluminium in senile plaques, most of them consisting of aggregates of β-amyloid peptides, and the development of NFT in the presence of aluminium provide some support to the association between Alzheimer disease and the presence of aluminium in the brain. However, the coincidental observation of these neuropathological features and aluminium in the brain cannot confirm the causal role of aluminium in Alzheimer disease.

2.3.4 Epidemiological studies

A randomized controlled trial assessed the acute effects of oral ingestion of a common aluminium compound on neuropsychological function. The study included three groups: 16 patients with a diagnosis of possible or probable Alzheimer disease and other dementias (scores 26–10 on the Standardized Mini-Mental State Examination [MMSE]); 17 age-matched controls (patients' caregivers); and 10 younger volunteers (family members, hospital employees). Aluminium hydroxide gel with citrate or placebo was administered over 3 consecutive days in a crossover

design, with a 3-week washout period between the two 3-day test sessions. The dose was adjusted for each individual in order to attain serum aluminium levels of 50–150 µg/l. A neuropsychological test battery was administered to the subjects on day 1 and again on day 3, at time 0 and 90 min after ingestion of the study preparation. Thirty-eight out of the 55 participants completed the protocol. There were no significant differences in neuropsychological test battery scores between active and placebo for any of the individual tests or for any of the groups examined. The mean concentration of aluminium in serum on day 3, 90 min after ingestion, was 294 µg/l (95% confidence interval [CI] 181–407 µg/l); a quarter of the subjects were within the targeted range of 50–150 µg/l, whereas 66% were above this range. The absence of neuropsychological effects after short-term exposure at elevated levels of aluminium in serum was unexpected. In some cases, the levels of aluminium in serum far exceeded about 60–200 µg/l, at which aluminium has been associated with cognitive effects in dialysis dementia (Molloy et al., 2007).

(a) Aluminium in drinking-water and Alzheimer disease, dementia and cognitive disorders

The relationships between aluminium and silica in drinking-water and the risk of cognitive decline, dementia and Alzheimer disease in elderly people were investigated in two cohorts in the regions of Gironde and Dordogne, south-west France. The cohort PAQUID (Personnes Âgées Quid) is a prospective population-based cohort of 3777 elderly subjects, aged 65 years or older at recruitment (1988–1989), followed regularly up to 2004 and at a 10-year follow-up. The Aluminium-Maladie d'Alzheimer (ALMA+) cohort included 400 subjects, aged 75 years and over at entry in 1999; thus, these subjects were expected to be comparable with the subjects seen at the 10-year follow-up of the PAQUID cohort. As the methods to assess the exposure and outcomes were very similar, the subjects of both cohorts were pooled for this analysis. Exposure to aluminium and silica in drinking-water at the geographical level was based on the information on tap water chemical analysis provided by the sanitary administration for 91 drinking-water areas (77 for PAQUID and 14 for ALMA+). For each area, a weighted mean of all measures of aluminium and silica was computed using results of analyses of drinking-water carried out by the sanitary administration. For the evaluation of the subjects' past exposure (at the geographical level), the history of the water distribution network over the previous 10 years was taken into account. Exposure at the individual level used information from a dietary questionnaire, which contained specific questions about the daily consumption of tap water and bottled water, including the brand, combined with the bottled water composition provided by the respective distributing companies. Assessment of intellectual functioning was based upon the MMSE score. Cases of dementia were detected by a two-step procedure: first, all participants underwent an interview and a psychometric evaluation with a trained psychologist who systematically completed a questionnaire designed to fulfil standardized criteria for dementia; then, subjects positive for these criteria were examined by a senior neurologist who confirmed the diagnosis. Subjects with a diagnosis of dementia at recruitment were excluded from the analysis. In total, 1925 subjects were available for the analysis of cognitive function, performed using a random effects linear regression model. The mean exposure to aluminium from drinking-water was 0.025 mg/day,

96% supplied by tap water. Cognitive decline was greater in subjects with a high daily aluminium exposure (≥0.1 vs <0.1 mg/day, $P = 0.001$). The interaction between aluminium exposure and time was not significant when the demented subjects were excluded, suggesting that cognitive decline with time was related to daily aluminium exposure only when it is associated with a dementia process. During the 15-year follow-up, 461 subjects out of 1677 were diagnosed with dementia or Alzheimer disease; the association with aluminium or silica exposure was assessed by the Cox proportional hazards model. The risk of dementia or Alzheimer disease was associated with daily exposure to aluminium: relative risk (RR) of 2.26 (95% CI 1.00–5.07) for aluminium exposure greater than or equal to 0.1 mg/day versus less than 0.1 mg/day or RR of 1.28 (95% CI 1.05–1.58) for a 0.1 mg/day increase (as a continuous variable). When aluminium exposure was categorized by quartiles, only the highest level (>0.1 mg/day) was significantly associated when compared with the bottom quartile (RR = 2.34, 95% CI 1.03–5.32). Contrary to aluminium, silica was inversely associated with dementia or Alzheimer disease (RR = 0.88, 95% CI 0.79–0.99, for a 10 mg/day increase). All these estimates were adjusted for age, sex, educational level, wine consumption and place of residence; the estimates for aluminium and silica exposure were mutually adjusted as well. Although the prospective design must be considered as a strength of the study, there is limited power owing to the low number of subjects with the highest level of exposure; only 13 subjects, which included 6 cases, had aluminium exposure greater than or equal to 0.1 mg/day. Although the estimates were adjusted for several potentially confounding factors, the possibility of residual confounding cannot be excluded, mainly due to the lack of information on aluminium exposure from foods, thought to contribute approximately 95% of oral exposure (Rondeau et al., 2009).

A previous analysis of the PAQUID cohort was carried out among 292 subjects, including 55 Alzheimer disease cases, who provided a blood sample at the 10-year follow-up visit. Logistic regression was used to assess the potential risk of Alzheimer disease associated with aluminium in drinking-water and carrying the C2 allele in the transferrin gene. Transferrin is the major transport protein for aluminium, and deficient binding of aluminium to transferrin may increase the unbound aluminium, which could cross the blood–brain barrier. The risk of Alzheimer disease was not associated with either C2 carrier status or interaction between the C2 allele and aluminium exposure. However, aluminium exposure modified the risk of Alzheimer disease associated with apolipoprotein E (ApoE) ε4; carriers of ε4 with aluminium exposure in drinking-water less than 0.1 mg/day had an odds ratio (OR) of 5.98 (95% CI 2.13–16.8) compared with an OR of 2.72 (95% CI 0.99–7.43) among ε4 carriers with an aluminium exposure in drinking-water greater than or equal to 0.1 mg/day (Rondeau et al., 2006).

The association of Alzheimer disease with aluminium in drinking-water was assessed in the Canadian Study of Health and Aging cohort. After a 10-year follow-up, 490 Alzheimer disease incident cases were ascertained among the 7155 subjects recruited in 1991–1992. Exposure assessment was based on residential history collected from the subjects and data on aluminium concentrations from water treatment plants. A statistically significant association between the risk of Alzheimer disease and aluminium was found using Cox models with aluminium as the continuous variable; no association was observed in analyses with a

categorical variable or after adjustment for autocorrelation in a two-stage model. This study provides equivocal evidence that higher aluminium drinking-water levels are associated with increased risk of Alzheimer disease (Boom, 2008).

Finally, an ecological study was carried out in the Biga Peninsula, located in north-west Turkey, in order to evaluate the potential of aluminium to influence cognitive function. The Kirazli region was included in the study, as it has water supplies characterized by high acidic content and aluminium levels. At the time of the study, 73 people out of the 201 inhabitants agreed to participate. The control group consisted of 164 subjects selected from the 921 inhabitants of another region in the same province. A neurology specialist administered an MMSE and performed a neurological examination, and a blood sample was collected from each participant. All of the subjects obtained their drinking-water from the groundwater networks in their regions. Water samples collected in both regions revealed a much higher concentration of aluminium in Kirazli (13–16 mg/l) than in the control region (0.005–0.010 mg/l); there were also much higher levels of iron, manganese, lead and zinc. Despite the differences in aluminium levels in their water supplies, no statistically significant difference was detected between the serum levels of aluminium of participants living in the two regions. No statistically significant difference was detected in the distribution of MMSE scores or the presence of neuropathy at examination between the two regions (Bakar et al., 2010).

(b) Dementia and aluminium in haemodialysis patients

Dementia may occur in the course of dialysis; however, the risk factors for dementia of uraemic patients remain unclear. One cross-sectional study aimed to determine the difference in nutritional status and the contents of several plasma elements in haemodialysis patients with or without dementia. Forty-five haemodialysis patients were divided into two groups: 25 patients without dementia and 20 patients with dementia. In addition, a control group of 20 healthy volunteers was included. Thirteen non-dementia patients and 11 patients with dementia were treated with aluminium hydroxide for controlling phosphate levels. Dementia patients had significantly higher levels of plasma aluminium (11.7 vs 7.79 µg/dl), and both groups had higher levels than controls (3.17 mg/dl). Dementia patients also had significantly higher levels of iron, copper and magnesium and lower levels of zinc. Furthermore, dementia patients also had a significant increase in the levels of MDA, an indicator of lipid peroxidation, and MDA was positively correlated with levels of aluminium and magnesium and with the copper/zinc ratio. On the basis of these findings, the authors concluded that chronic haemodialysis may lead to significant changes in the serum that increase the susceptibility of uraemia patients to oxidative stress and inflammation, which could be associated with the development of dementia (Guo et al., 2009).

(c) Oral exposure to aluminium and bone health

Low bone formation and patchy osteomalacia have been observed in patients on dialysis and those who are on total parenteral nutrition. Standard solutions for parenteral nutrition of infants contain significant aluminium concentrations; a randomized trial was conducted to compare their long-term effects on bone health

with those from solutions specially sourced for low aluminium content. The trial aimed to test the hypothesis that neonatal exposure to aluminium in standard parenteral nutrition solutions results in reduced bone mass during adolescence. In total, 227 preterm infants were recruited in Cambridge and Norwich, England: 112 were assigned to receive standard aluminium (SA) solution and 115 to low-aluminium (LA) solution. Data were collected for the neonatal course, and the participants were invited for follow-up after 15 years; 59 out of the 177 participants eligible for follow-up were actually seen, 26 fed with SA solutions and 33 with LA solutions. Among the 118 subjects not seen at the 15-year follow-up visit, 48 were untraceable, 48 did not reply to the invitation and 22 declined to attend; the distribution of the drop-outs was very similar in both groups. Dual-energy radiograph absorptiometry was used to measure bone mineral content (BMC), bone area and bone mineral density (BMD) of the lumbar spine, hips and whole body. Mean aluminium exposure was significantly higher for infants fed with SA solutions compared with those fed with LA solutions (means 21.3 and 3.0 µg/kg bw per day, respectively). Most bone density measurements tended to be higher in the LA group, but the only statistically significant differences were observed for lumbar spine BMC (mean ± SD: 44.9 ± 8.8 g vs 39.8 ± 6.5 g, $P = 0.02$) and lumbar spine bone area (40.5 ± 5.4 cm^2 vs 37.8 ± 3.7 cm^2, $P = 0.03$). The increase in lumbar spine BMC seemed to be attributable to a concomitant increase in bone size in the LA group, as no difference between groups in lumbar spine BMC was seen after adjusting for height, weight and lumbar spine bone area. For lumbar spine BMD, the means ± SD for the LA and SA groups were, respectively, 1.10 ± 0.12 g/cm^2 and 1.05 ± 0.15 g/cm^2 ($P = 0.17$). In a non-randomized analysis with the total aluminium exposure from parenteral nutrition as a continuous variable, aluminium exposure from parenteral nutrition was not a significant predictor of BMC at any site, after adjusting for relevant neonatal variables. However, when aluminium exposure was categorized using the median exposure (55 µg/kg bw) as a cut-off, children with high exposure had significantly lower (7.6%) hip BMC. The mechanism for long-term effects of aluminium on bone health is unclear. A direct toxic effect seems unlikely, because bone tissue will have been replaced more than once by age 15 years. Aluminium exposure might modify the responsiveness of bone such that, for example, children who are exposed to more aluminium form less bone for a given level of mechanical stimulus. This could explain the apparent site-specific effects. The long-term clinical significance of the observed effects of early aluminium exposure on bone mass at 15 years cannot currently be quantified, although these subjects were only 5–8 years from attaining peak bone mass (Fewtrell et al., 2009).

A case–control study was carried out in Upsala, Sweden, to examine whether the aluminium content of bone differs between controls and hip fracture cases with and without dementia, in particular in patients with Alzheimer disease. Cases were 103 patients with hip fracture (81 women, 22 men, mean age 73 years), among whom 49 had a diagnosis of dementia, including 16 with Alzheimer disease. The control group consisted of 69 patients (33 women and 36 men, mean age 58 years) admitted to the hospital for arthroplasty because of osteoarthrosis of the hip or because of high-energy femoral or tibial fracture. During the operations, bone biopsies from the trabecular bone of the proximal femur or tibia were taken with an aluminium-free instrument and were then introduced into an inductively coupled

mass spectrometer for measurement of their content of aluminium. All samples contained aluminium, at concentrations ranging from 58 to 13 300 ng/g dry weight. There was an exponential increase in the aluminium content of bone with age, with a statistically significant quadratic term of age in a model that included age in continuous form. No significant differences were detected in sex- and age-adjusted mean log-transformed aluminium contents between the controls and the hip fracture cases with dementia ($P = 0.72$) or without dementia ($P = 0.33$). When bone aluminium content was categorized by quartiles, there was no association with the risk of hip fracture once adjusted for age and sex. The most important finding in this study is the sharp increase in the aluminium content of bone with increasing age, but there was no association between this content and the risk of hip fracture, which is the most serious consequence of osteoporosis. Hip fracture cases with dementia showed aluminium concentrations in trabecular bone that were similar to those of hip fracture cases without dementia (Hellström et al., 2005).

2.3.5 Occupational exposure to aluminium

The International Agency for Research on Cancer (IARC) has concluded that there is sufficient evidence that certain exposures occurring during aluminium production cause cancer. Pitch volatiles, containing mainly polycyclic aromatic hydrocarbons, have fairly consistently been suggested in epidemiological studies as being possible causative agents. There is no evidence of increased cancer risk in non-occupationally exposed persons, and IARC did not implicate aluminium itself as a human carcinogen (IARC, 1984).

A few studies have addressed the potential effects of occupational exposure to aluminium on reproductive and neurobehavioural outcomes. One survey was carried out in a North American aluminium smelter plant where several adverse pregnancy outcomes had been reported. The participation rate for the survey was 85%: 621 workers participated, out of a total of 730 eligible, including 515 males and 106 females. Working in the laboratory was significantly associated with the occurrence of congenital anomaly. The congenital anomalies reported were three cases of trisomy (9, 18 and 21) as well as two renal, two musculoskeletal, one ocular, one cardiovascular and one genitourinary anomaly. The mean air concentration of aluminium in the laboratory was 2.8 mg/m^3 (range 0.07–8.3 mg/m^3, median 1.1 mg/m^3), far below the threshold limit value set by the American Conference of Governmental Industrial Hygienists of 10 mg/m^3. Given the diverse anomaly types, the absence of other adverse outcomes and the low exposure levels, the authors concluded that there is little evidence to suggest that the excess of congenital anomalies was due to workplace factors (Sakr et al., 2010).

A review with meta-analysis has summarized the evidence regarding the potential impact of occupational aluminium exposure on cognitive and motor performance. The final sample consisted of nine studies with 449 exposed and 315 reference subjects. Exposure originated from welding, smelting or electrolysis. Mean urinary aluminium concentrations ranged from 13 to 133 mg/l, with mean exposure duration ranging from 4.7 to 19.2 years. Six different neuropsychological tests were considered, and 10 performance variables were analysed: 7 of the variables pertained to aspects of attention, 2 to motor performance and 1 to constructional

performance. Almost all overall effect sizes indicated poorer performance of the exposed group, but a statistically significant result was found only for the digit symbol test (difference between the mean scores in exposed and control group –0.43, 95% CI –0.77 to –0.08). Performance of this test was negatively related to urinary concentrations of aluminium. Although this result suggests that the test for which a significant result was obtained might be a potential screening instrument for measuring aluminium-related changes in performance, 1 significant effect size out of 10 analyses could be a chance result. Uncertainties remain with respect to confounding, as the extent of confounding that has to be taken into account for at least some of the tests cannot be determined (Meyer-Baron et al., 2007).

The potential neurotoxic effects of aluminium have been analysed in two parallel longitudinal studies of aluminium welders in Germany. In the first study, 44 male aluminium welders in train and truck industries were compared with 37 assembly workers from the same enterprises; in the second study, 97 male aluminium welders in the automobile industry were compared with 50 non-exposed construction workers. In both studies, four examinations took place during a 4-year period; exposure was monitored by means of measurements of aluminium concentrations in the environment, as well as in urine and plasma of workers. The assessment of neurobehavioural performance included a questionnaire for the recording of neurotoxic symptoms and a number of psychological tests exploring different functional domains and premorbid intelligence. The aluminium welders who had been working in these industries for an average of 15 years showed no significantly increased symptom levels compared with the control group. The analyses revealed neither a correlation between biomonitoring and performance variables nor a significant difference between aluminium-exposed workers and controls in the performance courses during the 4-year period (Kiesswetter et al., 2007, 2009).

3. DIETARY EXPOSURE

3.1 Introduction

At the present meeting, the Committee was asked, based on the recommendation of the Forty-second Session of CCFA (FAO/WHO, 2010), to evaluate the safety of aluminium-containing food additives, including aluminium ammonium sulfate, aluminium lakes of colouring matters, aluminium potassium sulfate, aluminium powder, aluminium silicate, aluminium sulfate (anhydrous), calcium aluminium silicate, sodium aluminium phosphate acidic, sodium aluminium phosphate basic and aluminium lactate.

Owing to their multiple functions, aluminium-containing food additives are permitted for use in a large variety of foods. At its present meeting, the Committee was asked to evaluate the safety of potassium aluminium silicate–based pearlescent pigments based on the recommendation of the Forty-second Session of CCFA (FAO/WHO, 2010). This aluminium-containing food additive has not previously been evaluated by the Committee. All data necessary for the assessment of dietary exposure to the substance, including information on actual use levels, was

requested by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) in a July 2010 call for data.

At its present meeting, the Committee, following its call for data, received submissions from:

- the International Council of Grocery Manufacturer Associations (ICGMA, 2010) on the current use levels for aluminium sulfate (International Numbering System [INS] 520), sodium aluminosilicate (INS 554), sodium aluminium phosphate acidic (INS 541(i)) and aluminium lakes of colour;
- Food Standards Australia New Zealand (FSANZ, 2011) on dietary exposure to aluminium compounds in food, including additives, from its twenty-third Total Diet Study;
- Brazil (Aparecida, 2009) on the usages of aluminium-containing food additives and estimates of dietary exposure using the maximum permitted levels of these food additives.

Those submissions were complemented by a review of data from the literature published since the last JECFA evaluation in 2007 (accessible via Scopus or Medline as of 20 May 2011) from Europe (EFSA, 2008), the United Kingdom (Rose et al., 2010) and China, Hong Kong Special Administrative Region (SAR) (Wong et al., 2010).

A submission of data for the exposure assessment was provided to JECFA by a sponsor on 30 November 2010 (Merck, 2010). The exposure data provided by the sponsor were not for potassium aluminium silicate itself, but rather for the actual potassium aluminium silicate–based pearlescent pigment.

3.2 Use levels of the additives in food

3.2.1 Aluminium-containing food additives in the Codex General Standard for Food Additives

As shown in Table 3, three of the additives are currently listed in Table 3 of the Codex General Standard for Food Additives (GSFA) for use in most food categories at good manufacturing practice (GMP) levels; however, the Codex Alimentarius Commission revoked the Table 3 status for these three additives based on the recommendation of the Forty-third Session of CCFA (FAO/WHO, 2011).

All of the aluminium-containing food additives except aluminium lactate have previously been evaluated by the Committee.

(a) Current status of aluminium-containing food additives in the Codex General Standard for Food Additives

Because of their multiple functions, aluminium-containing food additives are permitted for use in a large variety of foods. The current (adopted, draft and proposed draft) provisions made for aluminium compounds in Tables 1 and 2 of the Codex GSFA are reported in Table 4. In addition to the revocation of the GMP status of three aluminium additives in Table 3 of the GSFA, the Forty-third Session of CCFA recommended discontinuation or revocation of all aluminium provisions

Table 3. Aluminium-containing food additives in the Codex General Standard for Food Additives

INS	Additive name	Relevant tables
523	Aluminium ammonium sulfate	GSFA Tables 1 and 2
541(i)	Sodium aluminium phosphate acidic	GSFA Tables 1 and 2
541(ii)	Sodium aluminium phosphate basic	
554	Sodium aluminosilicate	GSFA Tables 1, 2 and 3 (Table 3 GMP status revoked based on recommendation of the Forty-third Session of CCFA)
556	Calcium aluminium silicate	GSFA Tables 1, 2 and 3 (Table 3 GMP status revoked based on recommendation of the Forty-third Session of CCFA)
559	Aluminium silicate	GSFA Tables 1, 2 and 3 (Table 3 GMP status revoked based on recommendation of the Forty-third Session of CCFA)

that are listed without numerical use levels in Tables 1 and 2 of the Codex GSFA (highlighted in the final column of Table 4).

(b) Current use levels made available to the Committee by the International Council of Grocery Manufacturer Associations

The ICGMA (2010) submitted information on current use levels for aluminium lakes of colour, aluminium sulfate (INS 520), sodium aluminium phosphate acidic (INS 541(i)) and sodium aluminosilicate (INS 554) (Table 5).

3.2.2 Potassium aluminium silicate

The aluminium portion of the dietary assessment provided here refers only to aluminium from the food additive use of the potassium aluminium silicate–based pearlescent pigment and does not refer to aluminium as a contaminant present in foods as consumed. The contaminant portion of aluminium exposure has previously been assessed by the Committee at its sixty-seventh meeting in 2007 (Annex 1, reference *184*).

Potassium aluminium silicate (mica) is used as a carrier substrate for titanium dioxide and/or iron oxide. Potassium aluminium silicate is not intended to be placed on the market as such, but only when coated with the food colours titanium dioxide and/or iron oxide. In the European Union (EU), E555 potassium aluminium silicate is approved as a carrier for E171 titanium dioxide and E172 iron oxides and hydroxides (maximum 90% potassium aluminium silicate relative to the pigment) (Directive 95/2/EC as amended by Directive 2003/114/EC). In the

Table 4. List of all aluminium provisions in the Codex General Standard for Food Additives: adopted (Step 8), draft (Step 6) and proposed draft (Step 3)[a]

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
Aluminium ammonium sulfate (INS 523)						
01.1.2	Dairy-based drinks, flavoured and/or fermented (e.g. chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks)	350	6	3	—	—
01.7	Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	150	6	3	—	—
04.1.2.7	Candied fruit	200	6	8	2001	—
04.2.2.3	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweeds in vinegar, oil, brine or soya bean sauce	500	6	3	—	—
04.2.2.3	Vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweeds in vinegar, oil, brine or soya bean sauce	35	6	8	2003	—
04.2.2.6	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed pulps and preparations (e.g. vegetable desserts and sauces, candied vegetables) other than food category 04.2.2.5	200	6	8	2001	—
04.2.2.7	Fermented vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera) and seaweed products, excluding fermented soya bean products of food categories 06.8.6, 06.8.7, 12.9.1, 12.9.2.1 and 12.9.2.3	500	6	3	—	—

Table 4 (contd)

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
06.2	Flours and starches (including soya bean powder)	500	6	3	—	—
06.2.2	Starches	GMP	6 & 26	6	—	Discontinue
06.4.1	Fresh pastas and noodles and like products	470	6	3	—	—
07.1.2	Crackers, excluding sweet crackers	10 000	29	3	—	—
07.1.3	Other ordinary bakery products (e.g. bagels, pita, English muffins)	10 000	29	3	—	—
07.1.4	Bread-type products, including bread stuffing and bread crumbs	10 000	29	3	—	—
07.1.5	Steamed breads and buns	10 000	29	3	—	—
07.1.6	Mixes for bread and ordinary bakery wares	10 000	6	3	—	—
07.2	Fine bakery wares (sweet, salty, savoury) and mixes	10 000	29	3	—	—
08.3.2	Heat-treated processed comminuted meat, poultry and game products	5	6	3	—	—
09.2	Processed fish and fish products, including molluscs, crustaceans and echinoderms	1 500	6	3	—	—
09.2.4	Cooked and/or fried fish and fish products, including molluscs, crustaceans and echinoderms	200	6	8	2001	—
09.3	Semi-preserved fish and fish products, including molluscs, crustaceans and echinoderms	1 500	6	3	—	—
10.2	Egg products	30	6	8	2001	—
10.4	Egg-based desserts (e.g. custard)	380	6	8	2003	—

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
12.2	Herbs, spices, seasonings and condiments (e.g. seasoning for instant noodles)	500	6	3	—	—
15.1	Snacks: potato, cereal, flour or starch based (from roots and tubers, pulses and legumes)	500	6	3	—	—
Sodium aluminium phosphate acidic (INS 541(i)) and sodium aluminium phosphate basic (INS 541(ii))						
01.6.1	Unripened cheese	670	6	3	—	—
01.6.4	Processed cheese	35 000	29	6	—	—
01.7	Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	2 000	6	6	—	—
02.4	Fat-based desserts excluding dairy-based dessert products of food category 01.7	2 000	6	6	—	—
04.1.2.9	Fruit-based desserts, including fruit-flavoured water-based desserts	2 000	6	6	—	—
05.1.1	Cocoa mixes (powders) and cocoa mass/cake	2 000	6 & 72	6	—	—
05.2	Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	350	29	3	—	—
06.2	Flours and starches (including soya bean powder)	3 600	6	3	—	—
06.2.1	Flours	45 000	29	6	—	—
06.5	Cereal- and starch-based desserts (e.g. rice pudding, tapioca pudding)	2 000	6	6	—	—
06.6	Batters (e.g. for breading or batters for fish or poultry)	1 600	6	6	—	—
07.1	Bread and ordinary bakery wares	2 000	6	6	—	—
07.2.1	Cakes, cookies and pies (e.g. fruit-filled or custard types)	2 000	6	6	—	—

Table 4 (contd)

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
07.2.2	Other fine bakery products (e.g. doughnuts, sweet rolls, scones and muffins)	2 000	6	6	—	—
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	15 300	29	6	—	—
08.3.3	Frozen processed comminuted meat, poultry and game products	360	6	3	—	—
09.2.2	Frozen battered fish, fish fillets and fish products, including molluscs, crustaceans and echinoderms	190	6 & 41	6	—	—
09.2.4.3	Fried fish and fish products, including molluscs, crustaceans and echinoderms	600	6	3	—	—
10.4	Egg-based desserts (e.g. custard)	2 000	6	6	—	—
12.5.2	Mixes for soups and broths	2 000	6 & 127	6	—	—
12.6.3	Mixes for sauces and gravies	2 000	6 & 127	6	—	—
16.0	Composite foods: foods that could not be placed in categories 01–15	190	6	6	—	—
Sodium aluminosilicate (INS 554)						
01.1.2	Dairy-based drinks, flavoured and/or fermented (e.g. chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks)	20 000	6	3	—	—
01.3	Condensed milk and analogues (plain)	20 000	6	3	—	—
01.4.4	Cream analogues	20 000	6	3	—	—
01.5	Milk powder and cream powder and powder analogues (plain)	10 000	6 & 174	3	—	—
01.6.2.1	Ripened cheese, includes rind	10 000	6, 174 & 177	3	—	—

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
01.6.2.3	Cheese powder (for reconstitution; e.g. for cheese sauces)	10 000	6 & 174	3	—	—
01.6.4	Processed cheese	10 000	6, 174 & 177	3	—	—
01.6.5	Cheese analogues	10 000	6, 174 & 177	3	—	—
01.8.1	Liquid whey and whey products, excluding whey cheeses	20 000	6	3	—	—
01.8.2	Dried whey and whey products, excluding whey cheeses	10 000	6 & 174	3	—	—
01.8.2	Dried whey and whey products, excluding whey cheeses	10 000	—	8	2006	—
04.2.2.2	Dried vegetables (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweeds, and nuts and seeds	20 000	6	3	—	—
05.2	Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	GMP	3, 6 & 174	3	—	Discontinue
05.3	Chewing gum	GMP	3, 6 & 174	3	—	Discontinue
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	GMP	3, 6 & 174	3	—	Discontinue
06.3	Breakfast cereals, including rolled oats	20 000	6	3	—	—
06.4.3	Pre-cooked pastas and noodles and like products	2 0000	6	3	—	—
06.5	Cereal- and starch-based desserts (e.g. rice pudding, tapioca pudding)	20 000	6	3	—	—
06.6	Batters (e.g. for breading or batters for fish or poultry)	20 000	6	3	—	—
07.1.6	Mixes for bread and ordinary bakery wares	10 000	6 & 174	3	—	—

Table 4 (contd)

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	10 000	6	3	—	—
08.3	Processed comminuted meat, poultry and game products	GMP	6, 174 & 179	3	—	Discontinue
08.4	Edible casings (e.g. sausage casings)	GMP	3, 6 & 174	3	—	Discontinue
11.1.2	Powdered sugar, powdered dextrose	10 000	6 & 174	3	—	—
11.1.2	Powdered sugar, powdered dextrose	15 000	56	8	2006	—
12.1.1	Salt	20 000	6	3	—	—
12.1.1	Salt	GMP		8	2006	Revoke
12.1.2	Salt substitutes	10 000		6	—	—
12.2.2	Seasonings and condiments	30 000	6 & 174	3	—	—
12.5.2	Mixes for soups and broths	10 000	6 & 174	3	—	—
12.6.3	Mixes for sauces and gravies	10 000	6 & 174	3	—	—
13.6	Food supplements	GMP	6 & 174	3	—	Discontinue
14.1.4.3	Concentrates (liquid or solid) for water-based flavoured drinks	10 000	6 & 174	3	—	—
15.1	Snacks: potato, cereal, flour or starch based (from roots and tubers, pulses and legumes)	120	6	3	—	—
Calcium aluminium silicate (INS 556)						
01.5	Milk powder and cream powder and powder analogues (plain)	10 000	6 & 174	3	—	—
01.6.1	Unripened cheese	10 000	6 & 174	3	—	—
01.6.2.1	Ripened cheese, includes rind	10 000	6, 174 & 177	3	—	—

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
01.6.2.3	Cheese powder (for reconstitution; e.g. for cheese sauces)	10 000	6 & 174	3	—	—
01.6.4	Processed cheese	10 000	6, 174 & 177	3	—	—
01.6.5	Cheese analogues	10 000	6, 174 & 177	3	—	—
01.7	Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	10 000	6 & 174	3	—	—
01.8.2	Dried whey and whey products, excluding whey cheeses	265	6 & 174	3	—	—
01.8.2	Dried whey and whey products, excluding whey cheeses	10 000	—	8	2006	—
05.2	Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	GMP	3, 6 & 174	3	—	Discontinue
05.3	Chewing gum	GMP	3, 6 & 174	3	—	Discontinue
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	GMP	3, 6 & 174	3	—	Discontinue
06.1	Whole, broken or flaked grain, including rice	GMP	—	6	—	Discontinue
07.1.6	Mixes for bread and ordinary bakery wares	10 000	6 & 174	3	—	—
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	10 000	6 & 174	3	—	—
08.3	Processed comminuted meat, poultry and game products	GMP	6, 174 & 179	3	—	Discontinue
08.4	Edible casings (e.g. sausage casings)	GMP	3, 6 & 174	3	—	Discontinue
11.1.2	Powdered sugar, powdered dextrose	15 000	6 & 56	3	—	—
11.1.2	Powdered sugar, powdered dextrose	15 000	56	8	2006	—
12.1.1	Salt	20 000	6	3	—	—

Table 4 (contd)

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
12.1.1	Salt	GMP	—	8	2006	Revoke
12.1.2	Salt substitutes	10 000	—	6	—	—
12.2.2	Seasonings and condiments	30 000	6 & 174	3	—	—
12.5.2	Mixes for soups and broths	10 000	6 & 174	3	—	—
12.6.3	Mixes for sauces and gravies	10 000	6 & 174	3	—	—
13.6	Food supplements	GMP	6 & 174	3	—	Discontinue
14.2.3	Grape wines	GMP	—	6	—	Discontinue
Aluminium silicate (INS 559)						
01.5	Milk powder and cream powder and powder analogues (plain)	10 000	6 & 174	3	—	—
01.6.1	Unripened cheese	10 000	6	3	—	—
01.6.2.1	Ripened cheese, includes rind	10 000	6, 174 & 177	3	—	—
01.6.2.3	Cheese powder (for reconstitution; e.g. for cheese sauces)	10 000	6 & 174	3	—	—
01.6.4	Processed cheese	10 000	6, 174 & 177	3	—	—
01.6.5	Cheese analogues	10 000	6, 174 & 177	3	—	—
01.7	Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	10 000	6 & 174	3	—	—
01.8.2	Dried whey and whey products, excluding whey cheeses	10 000	6 & 174	3	—	—
01.8.2	Dried whey and whey products, excluding whey cheeses	10 000	—	8	2006	—
05.2	Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	GMP	3, 6 & 174	3	—	Discontinue

Food category No.	Food category name	Maximum level (mg/kg)	GSFA notes[b]	Step	Year adopted	CCFA decision
05.3	Chewing gum	GMP	3, 6 & 174	3	—	Discontinue
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	GMP	3, 6 & 174	3	—	Discontinue
06.1	Whole, broken or flaked grain, including rice	GMP	—	6	—	Discontinue
07.1.6	Mixes for bread and ordinary bakery wares	10 000	6 & 174	3	—	—
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	10 000	6 & 174	3	—	—
08.3	Processed comminuted meat, poultry and game products	GMP	6, 174 & 179	3	—	Discontinue
08.4	Edible casings (e.g. sausage casings)	GMP	3, 6 & 174	3	—	Discontinue
12.1.1	Salt	10 000	6	3	—	—
12.1.2	Salt substitutes	10 000	—	6	—	—
12.2.1	Herbs and spices	GMP	51	3	—	Discontinue
12.2.2	Seasonings and condiments	30 000	6 & 174	3	—	—
12.5.2	Mixes for soups and broths	10 000	6 & 174	3	—	—
12.6.3	Mixes for sauces and gravies	10 000	6 & 174	3	—	—
13.6	Food supplements	GMP	6 & 174	3	—	Discontinue

[a] GMP aluminium provisions were recommended for discontinuation at the Forty-third Session of CCFA.

[b] Relevant GSFA notes:

Note 3 Surface treatment.
Note 6 As aluminium.
Note 26 For use in baking powder only.
Note 29 Reporting basis not specified.
Note 41 Use in breading or batter coatings only.
Note 51 For use in herbs only.
Note 56 Provided starch is not present.
Note 72 Ready-to-eat basis.
Note 127 As served to the consumer.
Note 174 Singly or in combination: sodium aluminosilicate (INS 554), calcium aluminium silicate (INS 556) and aluminium silicate (INS 559).
Note 177 For use in sliced, cut, shredded or grated cheese only.
Note 179 For use in surface treatment of sausages.

Table 5. Current use levels of aluminium-containing food additives submitted by the International Council of Grocery Manufacturer Associations

(a) Aluminium lakes of colour

Food category No.	Food category name	Comments to CCFA		
		Concentration range of lake (mg/kg)	Reporting basis as aluminium (Note 6)[a]	Justification
01.6.2.3	Cheese powder (for reconstitution; e.g. for cheese sauces)	500	—	Powder, unreconstituted
01.7	Dairy-based desserts (e.g. pudding, fruit or flavoured yoghurt)	2.4–6.7	—	—
02.1.2	Vegetable oils and fats (e.g. popcorn oil)	1.2–215.5	—	—
04.2.2.6	Vegetable (including mushrooms and fungi, roots and tubers, pulses and legumes, and aloe vera), seaweed, and nut and seed pulps and preparations (e.g. vegetable desserts and sauces, candied vegetables) other than food category 04.2.2.5 (example – salsa)	600–20 000	80–4000 mg/kg as Al supplied	—
05.2.2	Soft candy (e.g. yoghurty coating around fruity-flavoured chewy centres)	—	5–200 mg/kg as Al supplied	—
05.3	Chewing gum	Up to 2000	—	—
05.4	Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	1.2–58.5	—	Icings, frostings
06.3	Breakfast cereals, including rolled oats (e.g. mini-wheats)	—	30 mg/kg as Al supplied	—

Food category No.	Food category name	Comments to CCFA		
		Concentration range of lake (mg/kg)	Reporting basis as aluminium (Note 6)[a]	Justification
06.4	Pastas and noodles and like products (e.g. rice paper, rice vermicelli, soya bean pastas and noodles)	800–8000	160–1600 mg/kg as Al supplied	—
07.2.1	Cakes, cookies and pies (e.g. fruit-filled or custard types) (e.g. cookies, pop-tarts)	290–1270	3–85 mg/kg as Al supplied	Pop-tarts
07.2.2	Other fine bakery products (e.g. doughnuts, sweet rolls, scones and muffins) (e.g. pancakes, waffles, snack bars)	—	1–50 mg/kg as Al supplied	Pancakes, waffles, snack breakfast bars
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	1.2–215	—	Cake mixes
12.2.1	Herbs and spices	900–10 000	125–1500 mg/kg as Al supplied	—
12.2.2	Seasonings and condiments	100–15 000	15–2900 mg/kg as Al supplied	—
12.6.2	Non-emulsified sauces (e.g. ketchup, cheese sauce, cream sauce, brown gravy)	39.7–2000	20–300 mg/kg as Al supplied	Cheese sauce
12.6.3	Mixes for sauces and gravies	100–95 000	15–1300 mg/kg as Al supplied	Meat marinade, as reconstituted
14.1.4.3	Concentrates (liquid or solid) for water-based flavoured drinks	—	1.6–14 mg/kg as Al (3–26 mg/kg as Al_2O_3)	These levels correspond to the reconstituted concentrate as consumed by the consumer (Note 127)[b] (If not reconstituted, then 148 mg/kg as Al_2O_3)

Table 5 (contd)

Food category No.	Food category name	Comments to CCFA		
		Concentration range of lake (mg/kg)	Reporting basis as aluminium (Note 6)[a]	Justification
15.1	Snacks: potato, cereal, flour or starch based (from roots and tubers, pulses and legumes) (e.g. chips, snacks)	300–800	100–400 mg/kg as Al supplied	Popcorn, snacks, trail mix, etc.
		300–48 000 (chips)	40–7200 mg/kg as Al supplied	

[a] Note 6: As aluminium.
[b] Note 127: As served to the consumer.

(b) Aluminium sulfate (INS 520)

Food category No.	Food category name	Comments to CCFA (Note 6)		
		Concentration range of lake (mg/kg)	Reporting basis as aluminium	Justification
10.2	Egg products	400–600	60–95 mg/kg as Al	Emulsifier: protein coagulation suppressant (crystallization inhibitor). The aluminium binds with egg proteins to help maintain protein solubility during the pasteurization/ heating process.

(c) Sodium aluminium phosphate acidic (INS 541(i); Function: acidity regulator, emulsifier, raising agent, stabilizer, thickener)

Food category No.	Food category name	Maximum level (mg/kg)	Comments[a]	Step[b]	Comments to CCFA		
					Maximum level (mg/kg)	Comments[a]	Justification
07.1	Bread and ordinary bakery wares	2000	Note 6	6	1000	Note 6	Sodium aluminium phosphate acidic is used as a leavening/raising agent to help with dough/texture formation. A maximum level of 11 000 mg/kg on the basis of the whole compound is necessary to achieve its intended function. The maximum level on the basis of aluminium would be 937 mg/kg. This is used in baking mixes for bread (Food category 07.1.1) and pizza crust (Food category 07.1.6), for example.
07.2.1	Cakes, cookies and pies (e.g. fruit-filled or custard types)	2000	Note 6	6	200	Note 6	Sodium aluminium phosphate acidic is used as a leavening/raising agent to help with dough/texture formation. A maximum level of 2220 mg/kg on the basis of the whole compound is necessary to achieve its intended function. The maximum level on the basis of aluminium would be 190 mg/kg. This is used in cookies and pop-tarts.
07.2.2	Other fine bakery products (e.g. doughnuts, sweet rolls, scones and muffins)	2000	Note 6	6	1000	Note 6	Sodium aluminium phosphate acidic is used as a leavening/raising agent to help with dough/texture formation. A maximum level of 11 000 mg/kg on the basis of the whole compound is necessary to achieve its intended function. The maximum level on the basis of aluminium would be 937 mg/kg. This is used in muffins, French toast, filled sweet rolls, waffles, Cinnabon, pancakes and baked wafers.

Table 5 (contd)

Food category No.	Food category name	Maximum level (mg/kg)	Comments[a]	Step[b]	Comments to CCFA		
					Maximum level (mg/kg)	Comments[a]	Justification
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	15 300	Note 6	6	1000	Note 6	Sodium aluminium phosphate acidic is used as a leavening/ raising agent to help with dough/texture formation. A maximum level of 11 000 mg/kg on the basis of the whole compound is necessary to achieve its intended function. The maximum level on the basis of aluminium would be 937 mg/kg. This is used in baking mixes for muffins, cakes and pancakes, for example.

[a] Note 6: As aluminium.
[b] Step 6: Draft.

(d) Sodium aluminosilicate (INS 554; Function: anticaking agent)

Food category No.	Food category name	Maximum level (mg/kg)	Comments[a]	Step[b]	Comments to CCFA		
					Maximum level (mg/kg)	Comments[a]	Justification
01.1.2	Dairy-based drinks, flavoured and/or fermented (e.g. chocolate milk, cocoa, eggnog, drinking yoghurt, whey-based drinks)	20 000	Note 6	3	100	Note 6	Sodium aluminosilicate is used in dry mix hot chocolate at levels of 1000 mg/kg on the basis of the whole compound and 57 mg/kg on the basis of aluminium.

Food category No.	Food category name	Maximum level (mg/kg)	Comments[a]	Step[b]	Comments to CCFA		
					Maximum level (mg/kg)	Comments[a]	Justification
01.3	Condensed milk and analogues (plain)	20 000	Note 6	3	1000	Note 6	Levels of 10 000 mg/kg on the basis of the whole compound (or 570 mg/kg on the basis of aluminium) are necessary for beverage whiteners (Food category 01.3.2), including non-dairy creamer powder and coffee whitener powder.
01.5	Milk powder and cream powder and powder analogues (plain)	10 000	Notes 6 & 174	3	1000	Note 6	Levels of 10 000 mg/kg on the basis of the whole compound (or 570 mg/kg on the basis of aluminium) are necessary for milk/cream powder analogues (e.g. soya oil powder), and levels of 5000 mg/kg on the basis of the whole compound (or 285 mg/kg on the basis of aluminium) are necessary for dairy-based creamers (e.g. milk powder and cream powder).
01.6.2.3	Cheese powder (for reconstitution; e.g. for cheese sauces)	10 000	Notes 6 & 174	3	1500	Note 6	Sodium aluminosilicate is an anticaking agent that helps prevent components from adhering to each other. To ensure flowability for the cheese powder and to prevent clumping, a maximum level of 25 000 mg/kg on the basis of the whole compound (or 1425 mg/kg on the basis of aluminium) is being recommended.
07.1.6	Mixes for bread and ordinary bakery wares	10 000	Notes 6 & 174	3	500	Note 6	Levels of use range from 0.1% to 0.6% (6000 mg/kg on the basis of the whole compound or 342 mg/kg on the basis of aluminium) and are necessary to prevent clumping and ensure flowability.

Table 5 (contd)

Food category No.	Food category name	Maximum level (mg/kg)	Comments[a]	Step[b]	Comments to CCFA		
					Maximum level (mg/kg)	Comments[a]	Justification
07.2.3	Mixes for fine bakery wares (e.g. cakes, pancakes)	10 000	Note 6	3	1500	Note 6	To ensure flowability and prevent clumping, levels of 20 000 mg/kg on the basis of the whole compound (or 1140 mg/kg on the basis of aluminium) are necessary.
12.2.2	Seasonings and condiments	30 000	Notes 6 & 174	3	2000	Note 6	Sodium aluminosilicate is required as an anticaking agent in seasonings to prevent clumping and improve flowability. Levels of 30 000 mg/kg on the basis of the whole compound (or 1710 mg/kg on the basis of aluminium) are necessary.
12.5.2	Mixes for soups and broths	10 000	Notes 6 & 174	3	1000	Note 6	Sodium aluminosilicate is required as an anticaking agent in these mixes to prevent clumping and improve flowability. Levels of 10 000 mg/kg on the basis of the whole compound (or 570 mg/kg on the basis of aluminium) are sufficient to carry out the intended function.
12.6.3	Mixes for sauces and gravies	10 000	Notes 6 & 174	3	1500	Note 6	Sodium aluminosilicate is required as an anticaking agent in these mixes to prevent clumping and improve flowability. Levels of 20 000 mg/kg on the basis of the whole compound (or 1140 mg/kg on the basis of aluminium) are sufficient to carry out the intended function.

Food category No.	Food category name	Maximum level (mg/kg)	Comments[a]	Step[b]	Comments to CCFA		
					Maximum level (mg/kg)	Comments[a]	Justification
15.1	Snacks: potato, cereal, flour or starch based (from roots and tubers, pulses and legumes)	120	Note 6	3	120	Note 6	Snacks frequently have seasoning mixtures applied to them to create new flavours of products. These seasonings must flow to properly adhere to the product. Sodium aluminosilicate is needed as an anticaking agent in these seasonings to prevent components from adhering to each other and then not adhering to the snack product. Necessary levels to achieve this function are 2000 mg/kg on the basis of the whole compound (or 114 mg/kg on the basis of aluminium).

[a] Note 6: As aluminium.
Note 174: Singly or in combination: sodium aluminosilicate (INS 554), calcium aluminium silicate (INS 556) and aluminium silicate (INS 559).
[b] Step 3: Proposed draft.

USA, pearlescent pigments consisting of potassium aluminium silicate coated with titanium dioxide are approved for use as a colour additive at levels up to 1.25% in cereals, confections and frostings, gelatine desserts, hard and soft candies (including lozenges), nutritional supplement tablets and capsules, and chewing gum (USFDA, 2006). Potassium aluminium silicate–based pearlescent pigments are proposed to be used in confectionery, chewing gums and beverages at usage levels ranging from a minimum of 0.02% up to a maximum of 1.25% (Table 6).

3.3 Estimates of dietary exposure

3.3.1 Aluminium-containing food additives

In its previous evaluation (Annex 1, reference *186*), the Committee considered only consumer exposure to aluminium in the diet; occupational exposure and other routes or commodities were not considered. Dietary sources of exposure include natural dietary sources, drinking-water, migration from food contact material and food additives. When dietary exposure was expressed on a kilogram body weight basis, a standard body weight of 60 kg for an adult was considered by the Committee, unless otherwise specified.

The Committee at its sixty-seventh meeting estimated mean exposure of the adult population from the overall diet, including additives, to range from 14 to 280 mg/week, expressed as aluminium.

In its conclusion, the Committee confirmed previous evaluations made by the Committee in which dietary exposure, particularly through foods containing aluminium compounds used as food additives, was found to represent the major route of aluminium exposure for the general population, excluding persons who regularly ingest aluminium-containing drugs.

(a) Screening by the budget method

The “budget method” is used to assess theoretical maximum daily dietary exposure. The budget method has been used as a screening method in assessing food additives by JECFA (FAO/WHO, 2001) and for assessments within the EU Scientific Cooperation (SCOOP) Task 4.2 (EC, 1998).

The method relies on assumptions regarding 1) the level of consumption of foods and of non-milk beverages, 2) the level of presence of the substance in foods and in non-milk beverages and 3) the proportion of foods and of non-milk beverages that may contain the substance. More specifically:

- The levels of consumption of foods and beverages considered are maximum physiological levels of consumption—i.e. the daily consumption of 0.1 litre/kg bw of non-milk beverages and the daily consumption of 100 kcal/kg bw from foods (equivalent to 0.05 kg/kg bw based on an estimated energy density of 2 kcal/g). In a person with a body weight of 60 kg, these levels correspond to the daily consumption of 6 litres of non-milk beverages and 3 kg of food (FAO/WHO, 2009).

Table 6. Proposed use levels for potassium aluminium silicate–based pearlescent pigments

Food categories Products coloured with titanium dioxide / iron oxide pearl effect colours based on potassium aluminium silicate (mica) as carrier	Corresponding food categories from the Codex GSFA	Reported use levels
Liquorice Pearl colours applied only on the product surface	5.2 Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.2–1.0%
Panned products (including hard sugar, chocolate and soft panned products) Pearl colour is applied only on the product surface	5.1 Cocoa products and chocolate products including imitations and chocolate substitutes	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.3–1.0%
Vermicelli, hundreds & thousands, nonpareils Pearl colour is applied only on the product surface	5.4 Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	Minimum efficacious level: 0.2% Maximum level: 1.25% Standard usage level: 0.4–1.0%
Jelly gums (transparent gums) Pearl colour is added to the product mass	5.2 Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.2–0.8%
Jelly gums (non-transparent gums) (i.e. wine gums) Pearl colour is applied on the product surface	5.2 Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.2–0.8%
Marzipan Pearl colour is applied on the product surface	5.2 Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.2–0.8%
Hard boiled candies/lollipops Pearl colour is applied on the product surface	5.2 Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.2–0.8%

Table 6 (contd)

Food categories Products coloured with titanium dioxide / iron oxide pearl effect colours based on potassium aluminium silicate (mica) as carrier	Corresponding food categories from the Codex GSFA	Reported use levels
Pearl colour is added to the product mass		Minimum efficacious level: 0.04% Maximum level: 1.25% Standard usage level: 0.06–0.8%
Compressed sweets (including lozenges & pastilles) Pearl colour is applied on the product surface	5.2 Confectionery including hard and soft candy, nougats, etc. other than food categories 05.1, 05.3 and 05.4	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.4–1.0%
Frostings Pearl colour is added only to the frosting	5.4 Decorations (e.g. for fine bakery wares), toppings (non-fruit) and sweet sauces	Levels refer to the final product on which the frosting or coating is applied: Minimum efficacious level: 0.4% Maximum level: 1.25% Standard usage level: 0.6–1.0%
Chewing gums Pearl colour is applied on the product surface	5.3 Chewing gum	Minimum efficacious level: 0.1% Maximum level: 1.25% Standard usage level: 0.3–1.0%
Beverages Beverages should be transparent in order to be suited for the application of pearl effect colours; they can be either carbonated or non-carbonated	14.2 Alcoholic beverages, including alcohol-free and low-alcoholic counterparts	Minimum efficacious level: 0.02% Maximum level: 0.5% Standard usage level: 0.08–0.3%
Beverages Water-based beverages have to be transparent in order to be suited for the application of pearl effect colours; they can be either carbonated or non-carbonated	14.1.4 Water-based flavoured drinks, including “sport”, “energy” or “electrolyte” drinks and particulated drinks	Minimum efficacious level: 0.02% Maximum level: 0.5% Standard usage level: 0.08–0.3%

Table 7. Theoretical maximum daily exposure to aluminium-containing food additives included in the Codex General Standard for Food Additives and for which uses have been identified in the International Council of Grocery Manufacturer Associations submission

INS	Food additive name	Maximum concentration submitted by ICGMA (mg/kg)[a]	Theoretical maximum daily exposure to aluminium (mg/kg bw per day)
—	Aluminium lakes of colour	Solid food: 4000 Liquid food: 14	25
520	Aluminium sulfate	95	0.6
541(i)	Sodium aluminium phosphate acidic	1000	6.2
554	Sodium aluminosilicate	1500	9

[a] Maximum level of use expressed on the basis of aluminium.

- The level present in foods is assumed to be the highest maximum level of the additive reported in any representative category, respectively, for foods and for beverages for all aluminium-containing food additives with current GSFA provisions.
- The proportion of, respectively, solid foods and beverages that may contain the substance is set generally at 12.5% and 25%. For these compounds, there are no provisions for non-milk beverages; therefore, the overall theoretical maximum daily exposure to each aluminium-containing food additive is calculated from the potential exposure from solid foods only.

Table 7 summarizes the maximum concentrations submitted by ICGMA for aluminium-containing food additives in the GSFA that are taken into account in the budget method for calculating the theoretical maximum daily dietary exposure to these additives. As non-milk beverages were not proposed as a food use category in ICGMA submissions except for aluminium lakes of colour, only solid foods were taken into account in the budget method calculation. The maximum levels of use provided by ICGMA were expressed both for the whole compounds and on the basis of aluminium. For the purpose of the evaluation, which refers to aluminium exposures, solely the maximum levels of use expressed on the basis of aluminium were used.

The Committee noted that no actual usage data (GSFA provisions or data submissions) were submitted for aluminium ammonium sulfate (INS 523), sodium aluminium phosphate basic (541(ii)), aluminium silicate (INS 559), aluminium powder or aluminium potassium sulfate (INS 522). As no uses were reported in the GSFA provisions for some of these food additives and no actual use levels were submitted by ICGMA for the same additives, the Committee concluded that these compounds have no usages in food categories currently reported and did not perform budget method calculations for these compounds.

The Committee noted that for other compounds evaluated at this meeting for which actual use levels were defined by ICGMA, the theoretical maximum dietary exposure based on the budget method is greater than the PTWI of 1 mg/kg bw for all aluminium-containing food additives except aluminium sulfate. Detailed assessment of the dietary exposure was therefore needed for those aluminium-containing food additives with theoretical maximum dietary exposures higher than the PTWI when used in solid foods and beverages.

(b) Concentrations of aluminium in foods and beverages and estimated national dietary exposures

Since the last aluminium evaluation (Annex 1, reference *186*), the Committee has reviewed new publications or submissions from Australia (FSANZ, 2011), Brazil (Aparecida, 2009), China (Wu, 2011), China, Hong Kong SAR (Wong et al., 2010), Europe (EFSA, 2008), Japan (Aung, Yoshinaga & Takahashi, 2006), Spain (González-Weller et al., 2010), the United Kingdom (Rose et al., 2010) and the USA (Saiyed & Yokel, 2005).

(i) Australia

The Australian permissions for use of aluminium-containing food additives are set out in Standard 1.3.1 – Food Additives of the Australia New Zealand Food Standards Code. The forms of aluminium-containing food additives of interest that are permitted in the Code, generally at GMP levels within each specified Australia New Zealand Food Classification System food group, are sodium aluminium phosphate (INS 541), potassium aluminium silicate (INS 555), aluminium silicate (INS 559), calcium aluminium silicate (INS 556), aluminium lakes of colouring matters and aluminium (INS 173). There are currently no permissions in Standard 1.3.1 – Food Additives to use the following forms of aluminium-containing food additives: aluminium ammonium sulfate, aluminium potassium sulfate, aluminium powder, aluminium sulfate, sodium aluminium phosphate and aluminium lactate.

FSANZ submitted the results of a dietary exposure assessment for aluminium from naturally occurring and food additive sources. Concentration data in food as consumed were analysed as part of the twenty-third Australian Total Diet Study (to be published in 2011; FSANZ, 2011). The dietary exposure assessment used food consumption data from two Australian National Nutrition Surveys: the 2007 Australian Children's Nutrition and Physical Activity Survey for children aged 2–16 years, which included two 24-hour recalls for all respondents, and the 1995 National Nutrition Survey for those aged 17 years and above, which included one 24-hour recall for all respondents.

Estimated dietary exposures to aluminium at the mean and 90th percentile (mg/day) were provided for all populations using median contaminant concentrations. Highest concentration levels were found in cereal products (from 2.2 mg/kg in breakfast cereal to 41.2 mg/kg in bread up to 108 mg/kg in chocolate cake; fish (from 19 to 25 mg/kg); and meat products (from 1.3 mg/kg in chicken to 15.7 mg/kg in beef sausages).

The estimated daily dietary exposure to aluminium for adult consumers was 0.04 mg/kg bw at the mean and 0.07 mg/kg bw for high-level consumers (90th percentile). For children, estimated daily dietary exposure to aluminium ranged from 0.03 mg/kg bw in teenagers (13–16 years) to 0.07 mg/kg bw in toddlers (2–5 years) at the mean and from 0.06 mg/kg bw to 0.15 mg/kg bw for the same two age groups of high-level consumers, respectively.

The major foods contributing to overall dietary exposure were tea for adults (35%); cakes, muffins and puddings (23% in adults to 38% in young children); white, multigrain, wholemeal and rye breads (13% in toddlers); and sausages and frankfurters (7% in toddlers).

(ii) Brazil

Aluminium-containing food additives authorized in the Mercosur harmonized list include sodium aluminium sulfate (INS 521), potassium aluminium sulfate (INS 522), aluminium ammonium sulfate (INS 523), sodium aluminium phosphate acidic (INS 541(i)), sodium aluminium phosphate basic (INS 541(ii)), sodium aluminosilicate (INS 554), calcium aluminium silicate (INS 556) and aluminium silicate (INS 559).

Among 1081 products investigated in the Aparecida (2009) study, only 2.8% presented aluminium salts on their labels. Among identified additives, sodium aluminosilicate (INS 554) and sodium aluminium phosphate acidic (INS 541(i)) were declared as anticaking and leavening agents, respectively.

The contribution of food additives as a source of aluminium in the Brazilian diet was estimated based on the consumption of foods that may contain aluminium salts combined with the maximum permitted levels of these additives. When appropriate, information provided by the industry as well as provisions under discussion in the Mercosur were used in the calculations. Consumption data were inferred from a household economic survey or taken from the Nutrition Facts label. Products for which aluminium-containing food additives are allowed were first identified from regulations in force, and then the list of ingredients used in each product was checked at the web site of a supermarket. The exposure to these additives from the consumption of condiments, seasonings, salt and mixes for soup, cereal products, bakery products and cookies and the exposure to aluminium lakes of colour from confectioneries (pastilles) were estimated using the theoretical maximum intake approach. The dietary exposures were calculated for both adults and children.

Estimated exposures to aluminium from sodium aluminosilicate corresponded to 47% and 95% of the PTWI of 1 mg/kg bw established by JECFA for aluminium from all sources for adults and children, respectively. In these estimates, table salt was the main contributor to the exposure to aluminium (20.8 mg/week).

Regarding sodium aluminium phosphate acidic, the exposure of children to aluminium corresponded to 1.38 mg/kg bw per week, with bread contributing 50% of this exposure (24.1 mg/week). For adults, exposures up to 2.9 mg/kg bw per week were observed in regions where the consumption of flour is high. Among all foods analysed in this study, cake was identified as the major potential source of exposure of children to aluminium (up to 15.7 mg/kg bw per week), followed by

confectioneries, which can contribute up to 1.8 mg of aluminium per kilogram of body weight per week. Although these results may be overestimates, owing to the conservative approach undertaken, it should be emphasized that the estimates did not take account of other dietary sources of aluminium, such as drinking-water, natural occurrence and migration from food contact materials (e.g. containers, cookware, utensils and packaging).

The authors recommended that regulatory agencies adopt measures to reduce the exposure of the population to aluminium, including the revision of the present legislation towards reducing or banning permitted use levels of aluminium salts. To allow a more realistic exposure estimate, food industries should provide data on the actual use levels of aluminium-containing additives while seeking alternative additives to replace them.

(iii) China

China submitted levels of aluminium in foods using the fourth (2007) Chinese Total Diet Study samples (Wu, 2011). Aluminium concentrations were obtained for 144 composite diet samples collected in 2007 from 12 provinces in China. Samples were analysed using the inductively coupled plasma mass spectrometry (ICP-MS) technique. The LOD was 2.0 µg/kg. The highest contributing food groups together with the mean aluminium concentrations in those groups were aquatic foods from Fujian (42.7 mg/kg), cereals from Hebei (39.0 mg/kg), cereals from Heilongjiang (27.6 µg/kg) and potatoes from Liaoning (26.9 µg/kg). The highest contributing food items together with the mean aluminium concentrations in those items were fritters from Heilongjiang (1242 mg/kg), Hebei (1025 mg/kg) and Liaoning (956 mg/kg), jiang doufu from Hebei (2423 mg/kg), starch noodles from Hebei (681 mg/kg), steamed bread from Guangxi (332 mg/kg) and Shanghai (208 mg/kg), wheat noodles from Hubei (301 mg/kg) and cake from Sichuan (260 mg/kg).

China submitted estimates of dietary exposure to aluminium based on the results of the 2007 Chinese Total Diet Study (Wu, 2011). Aluminium concentrations of 144 food composites from 665 food samples prepared as consumed were used in the calculations. Dietary exposure calculations were performed using a deterministic method, combining mean aluminium concentrations from the food group composites with their associated food consumption. Concentration values reported below the reporting limits were assigned a concentration equal to one half the LOD. In 2007, the fourth Chinese Total Diet Study included 4320 persons and covered four baskets from 12 provinces, municipalities and autonomous regions in mainland China. The average exposure estimate for the whole population was 13.0 mg/day, with a range between 3 and 19 mg/person per day. Main food contributors to total exposure were cereals (72.7%), vegetables (9.8%) and potatoes (5%). The average aluminium exposure by age group in China ranged between 2.7 mg/kg bw per week for those older than 65 years and 5 mg/kg bw per week for children 2–7 years of age. The 90th percentile aluminium dietary exposure ranged between 4.4 and 10 mg/kg bw per week for the same population groups, respectively.

In China, aluminium-containing food additives are allowed to be used in the process of making wheat and starch products, which makes this the main contributor to the overall exposure. The purpose of adding the aluminium-containing

food additives while making cereal products is to give the final products a bulky appearance and soft texture. With respect to the estimated data from the Chinese Total Diet Study, only about 10% of residents who loved eating fritters or starch noodles might have a risk of higher aluminium exposure.

(iv) China, Hong Kong Special Administrative Region

An assessment of dietary exposure to aluminium for the population of Hong Kong SAR was published by Wong et al. (2010). In this study, 256 individual food samples were collected from various locations in Hong Kong SAR for aluminium testing. Basically, for packaged products, only food items labelled with aluminium-containing food additives in the ingredient list were selected for the testing of aluminium, given that most of food samples were analysed in ready-to-eat form.

High aluminium levels were found in steamed bread/buns/cakes (mean: 100–320 mg/kg), some bakery products, such as muffins, pancakes/waffles, coconut tarts and cakes (mean: 250, 160, 120 and 91 mg/kg, respectively) and jellyfish (ready-to-eat form) (mean: 1200 mg/kg). The authors concluded that results demonstrated that aluminium-containing food additives have been widely used in these food products.

Estimates of dietary exposure were made using the average consumption data of the corresponding food type analysed from the Hong Kong SAR adult dietary survey of 1995. The average dietary exposure to aluminium from packaged food consumption products reported in the national dietary survey was estimated to be 0.60 mg/kg bw per week for a 60 kg adult, corresponding to 60% of the PTWI of 1 mg/kg bw.

The main dietary food additive source was “steamed bread/buns/cakes”, which contributed 0.4 mg/kg bw per week to the total exposure, followed by “bakery products” and “jellyfish”, with 0.1 mg/kg bw per week, respectively.

The authors noted that the estimation did not include the exposure to aluminium from natural food sources, food contact materials or other sources (e.g. drinking-water) and indicated that even if aluminium is unlikely to cause adverse health effects for the general population, the risk to some populations who regularly consume foods with aluminium-containing food additives cannot be excluded.

(v) Europe

In its scientific opinion on the safety of aluminium from dietary exposure, EFSA (2008) concluded that most unprocessed foods typically contain less than 5 mg of aluminium per kilogram. Higher concentrations (mean levels 5–10 mg/kg) were often found in breads, cakes and pastries (with biscuits having the highest levels), some vegetables (with mushrooms, spinach, radishes, swiss chard, lettuce and corn salad having the highest levels), glacé fruits, dairy products, sausages, offal, shellfish, sugar-rich foods, baking mixes and a majority of farinaceous products and flours. Foods with very high mean concentrations included tea leaves, herbs, cocoa and cocoa products, and spices. No analytical studies in Europe have focused on the aluminium content of food that contains permitted aluminium-containing food additives.

Total dietary exposure to aluminium from all sources has been estimated from duplicate diet studies (Germany, Hungary, Italy, the Netherlands and Sweden) and market basket and total diet studies (Finland, France and the United Kingdom). Mean dietary exposure from water and food in non-occupationally exposed adults showed large variations between the different countries and, within a country, between different surveys. It ranged from 1.6 to 13 mg of aluminium per day, corresponding to 0.2–1.5 mg/kg bw per week in a 60 kg adult. Children generally have higher food intake than adults when expressed on a body weight basis and therefore represent the group with the highest potential exposure to aluminium per kilogram body weight. Large individual variations in dietary exposure to aluminium can occur. In young people, the potential estimated exposure at the 97.5th percentile ranged from 0.7 mg/kg bw per week for children aged 3–15 years in France to 2.3 mg/kg bw per week for toddlers (1.5–4.5 years) and 1.7 mg/kg bw per week for those aged 4–18 years in the United Kingdom. The main contributors to overall mean dietary exposure were cereals and cereal products (50% in the United Kingdom and 20% in France), vegetables (20% in France) and beverages (30% in the United Kingdom).

Because of the design of the human dietary studies and the analytical methods used, which determine only the total aluminium content in food (from duplicate diet studies or total diet studies), and not the individual aluminium compounds or species present, it is not possible to conclude on the specific sources contributing to the aluminium content of a particular food, such as the amount inherently present, the contributions from use of food additives and the amounts released to the food during processing and storage from aluminium-containing foils, containers or utensils. Therefore, these contributions may also partly reflect the use of aluminium-containing food additives that are permitted for use (e.g. in some bakery products and aluminium from food colours used as aluminium lakes). Thus, a detailed breakdown by exposure source was not possible in the EFSA evaluation, but the panel nevertheless recognized that the major route of exposure to aluminium for the general population was through food.

(vi) Japan

The duplicate diet samples published by Aung, Yoshinaga & Takahashi (2006), including drinking-water, snacks and beverages, were collected from 33 households in November and December 2000 on 7 consecutive days. Nineteen out of 33 households were located in the city centre; the remaining households were in the suburb regions of Tokyo Metropolitan Area.

Exposures to aluminium were calculated for the individual subjects by multiplication of the weights of foods by the concentrations of aluminium in the samples and expressed in milligrams per kilogram of fresh material. The daily exposure ranges were calculated by averaging 7-day duplicate diet composites. The weekly average exposures to aluminium were estimated to be 2.85 mg/kg bw in children 3–6 years of age and 1.37 mg/kg bw in adults 28–40 years of age.

(vii) Spain

A dietary exposure assessment for aluminium in a Spanish population (Canary Islands) was published by González-Weller et al. (2010). The aim of

this study was to analyse the aluminium content in foods and beverages most commonly consumed by the population of the Canary Islands and to determine the dietary exposure to this metal throughout the Canary Islands as a whole and in each of the seven islands (Gran Canaria, Lanzarote, Fuerteventura, Tenerife, La Palma, La Gomera and El Hierro). Four hundred and forty samples collected over 28 months (2006–2008) in different shopping malls and representing the foods most commonly bought and consumed by the population of the Canary Islands were analysed by inductively coupled plasma optical emission spectrometry (ICP-OES). The highest average aluminium contents were found in vegetables (squash, carrots, bubang, cabbage, watercress, spinach: 27.5 mg/kg for the whole group), fruits (bananas: 32.8 mg/kg; peaches, pears, plums: 9.7 mg/kg), sweet cakes (14.2 mg/kg), viscera (11.2 mg/kg), red meat (9.3 mg/kg), poultry and rabbit (6.4 mg/kg), potatoes (5.9 mg/kg) and pastries (muffins, croissants, doughnuts, other bakery products: 5.7 mg/kg). Other food groups were generally found to contain aluminium at levels below 5 mg/kg.

The estimated average total exposure to aluminium for the population of the Canary Islands was 10.2 mg/day. In all of the islands, fruits and vegetables were found to be the food groups that contributed the most to the total average dietary exposure to aluminium.

(viii) United Kingdom

An assessment of dietary exposure to metals and other elements in the 2006 United Kingdom Total Diet Study and some trends over the last 30 years was published by Rose et al. (2010). The foods making up the 20 groups were bought from retail outlets in 24 randomly selected towns throughout the United Kingdom. The food samples were prepared and cooked according to normal consumer practices. Equal quantities of samples from each town were mixed for each food group to obtain the national composite samples. These composite samples for each food group were homogenized and supplied frozen at –20 °C for laboratory analysis.

Consumption data from the 1999 and 2002 British National Diet and Nutrition Survey were used to estimate dietary exposures for individuals in the general population who eat average amounts of each food group (i.e. consumers with mean consumption) and those who eat significantly more than average amounts (i.e. consumers with high-level, 97.5th percentile consumption). Total consumer dietary exposures are derived from an average of the individual consumer's exposure patterns with regard to individual foods.

In the 20 food groups of the 2006 Total Diet Study samples, most groups had aluminium concentrations lower than or similar to those reported in the 2000 Total Diet Study, the exceptions being bread, meat products and other vegetable groups. The miscellaneous cereals group had the highest concentration of aluminium (17.5 mg/kg, range from 4.8 to 78 mg/kg).

The population dietary exposure to aluminium was 5.4 mg/day, which was higher than the estimates from the 2000 Total Diet Study and the 1997 Total Diet Study (4.7 and 3.4 mg/day, respectively).

The estimated daily dietary exposure to aluminium was 0.07 mg/kg bw for adult consumers at the mean and 0.14 mg/kg bw for high-level consumers (97.5th percentile). For toddlers (1.5–4.5 years old) and young people (4–18 years old), estimated daily dietary exposure to aluminium was 0.19 mg/kg bw and 0.12 mg/kg bw at the mean and 0.35 mg/kg bw and 0.25 mg/kg bw for high-level consumers (97.5th percentile), respectively. The authors noted that estimates of high-level dietary exposure of toddlers, young people, the elderly and vegetarians exceeded the PTWI of 1 mg/kg bw by up to 2.4-fold. The major foods contributing to overall dietary exposure of the total population were miscellaneous cereals (flour, buns, cakes, pastries, chocolate biscuits and other biscuits, 42%), beverages (tea, instant coffee, cocoa, concentrated and ready-to-drink soft drinks, 34%) and bread (white, wholemeal, brown, 7%).

(ix) United States of America

A survey of the aluminium content of some foods and food products containing aluminium-containing food additives in the USA was published by Saiyed et al. (2005). The primary objective was to focus on processed food products in the USA and to determine the aluminium content of the selected foods that contain aluminium as an approved food additive.

Approximately 95 single-sample purchases were made locally during 2003–2004. The products purchased were nationally available brands and house brands that are presumed to be available nationally. They were selected to be representative of products that did, or did not, have added aluminium, according to the Nutrition Facts label. The purchase locations were varied, but as all the products were national brands or house brands of national chains, it was assumed that the products would be the same if purchased elsewhere. Some were known to contain aluminium from their list of ingredients. The aluminium-contributing ingredients, according to the products' Nutrition Facts labels, were sodium aluminium phosphate (INS 541) and sodium aluminosilicate (INS 554).

Frozen pizza that listed sodium aluminium phosphate as an additive generally had about 200–750 mg of aluminium per kilogram cheese. The cheese from frozen pizza products that did not list aluminium as an additive and ready-to-eat pizza had only a few milligrams of aluminium per kilogram, similar to a natural cheese, which contains about 0.5–3 mg of aluminium per kilogram.

The crust (grain product/bread) of frozen pizza that did not list aluminium as a food additive and of ready-to-eat pizza contained about 12 mg aluminium per kilogram, whereas a frozen pizza listing aluminium as an additive (sodium aluminium phosphate) in the crust had about 200 mg aluminium per kilogram. The high concentration of aluminium in the crust of the pizza containing aluminium as an additive is consistent with baked goods representing the largest source of aluminium in the typical diet.

The authors noted that the finding of considerable amounts of added aluminium in grain-based foods, particularly those containing self-rising flour, was in agreement with other publications. The very high aluminium concentration in baking powder is also consistent with previous reports of 20 000–34 000 mg aluminium

per kilogram. Considerable aluminium concentrations were found in many pancake and waffle mixes, frozen products and ready-to-eat products, up to 1200, 600 and 1200 mg/kg, respectively.

Exposure to aluminium from the labelled serving size of each food product was calculated. Food product aluminium content ranged from less than 1 to 27 000 mg/kg. Cheese in a serving of frozen pizzas had up to 14 mg of aluminium, from sodium aluminium phosphate basic, whereas the same amount of cheese in a ready-to-eat restaurant pizza provided 0.03–0.09 mg. Many single-serving packets of non-dairy creamer had 50–600 mg aluminium per kilogram as sodium aluminosilicate, providing up to 1.5 mg aluminium per serving. Many single-serving packets of salt also had sodium aluminosilicate as an additive, but the aluminium content was less than in single-serving non-dairy creamer packets. Sodium aluminium phosphate acidic was present in many food products, pancakes and waffles. Baking powder, some pancake and waffle mixes, some frozen products and ready-to-eat pancakes provided the most aluminium of the foods tested, up to 180 mg/serving.

The authors concluded that many products provide a significant amount of aluminium compared with the typical exposure of 3–12 mg/day reported from dietary aluminium studies conducted in many countries.

(x) Summary of national estimates of exposure

For aluminium-containing food additives under re-evaluation, a tentative estimate of dietary exposure from food additive sources has been made, taking into account previous assessments and other publications or submissions reviewed by the Committee at the current meeting. The Committee noted, from the report of its sixty-seventh meeting and from an EFSA scientific opinion, that the range of estimates was mainly based on dietary exposure calculated with the total diet study method, which takes into account water consumption. It is known from the literature that the main sources of migration of aluminium into food are from the use of cookware or aluminium utensils. It is also known that the design of total diet studies generally tries to control any bias of additional contamination that may result from the use of containers, cookware or utensils containing aluminium during the preparation and storage of food as consumed.

The Committee noted that estimates of the contribution to overall mean dietary exposure from all sources (including natural sources, water consumption, food contact materials and food additives) were in the range of 10–140 mg/week in adult populations (0.2–2.3 mg/kg bw per week as aluminium, assuming a body weight of 60 kg; Table 8). Major contributors to these estimates were cereals and cereal-based food products, with a proportion of 20–90%, depending on the country, equivalent to an exposure of approximately 2–120 mg/week (0.03–2 mg/kg bw per week as aluminium, assuming a body weight of 60 kg).

This assessment is consistent with previous evaluations made by the Committee in which cereal products were considered as potentially high contributors to dietary aluminium exposure. The Committee also noted from its review that high levels of the actual uses of aluminium-containing food additives were reported for cereals and cereal-based products, in particular for sodium aluminosilicate (INS 554)

Table 8. Estimated ranges of mean exposure of the adult population to aluminium from different dietary sources

Country/region	Estimated mean exposure (mg/person per week)	
	From food additives used in cereals and cereal-based products	From overall diet, including natural sources, water consumption, food contact materials and food additives
JECFA[a]	—	14–280
JECFA[b]	2–124	11–136
Australia	4	17
Brazil	40–70	—
China	4–124	23–136
China, Hong Kong SAR	30	36
Europe (EFSA)	2–46	11–91
Japan	—	84
USA	24–30	60

[a] Estimated ranges from the sixty-seventh meeting of the Committee (Annex 1, reference *186*).
[b] Estimated ranges from the data reviewed at this meeting.

and sodium aluminium phosphate acidic (INS 541(i)). Based on this, the Committee concluded that aluminium from the consumption of cereals and cereal-based products could reasonably be assumed to be mainly from food additive sources.

The Committee noted that the estimated dietary exposures related to average adult populations and that high dietary exposures (e.g. 90th or 95th percentile) are generally assumed to be 2 times higher than the reported average. It also noted that children generally have higher food intake than adults when expressed on a body weight basis and therefore represent the highest potential exposure to aluminium per kilogram of body weight.

(c) International estimates of dietary exposure

The Committee considered it inappropriate to use the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diets to calculate international estimates of dietary exposure because the aluminium-containing food additives are present mainly in prepared foods and not in staple foods.

3.3.2 Potassium aluminium silicate

(a) Annual poundage of the additive introduced into the food supply

The sponsor indicated that annual poundage data for the worldwide use of potassium aluminium silicate–based pearlescent pigments are not available. The

Table 9. Theoretical maximum daily exposure to potassium aluminium silicate–based pearlescent pigments for which uses have been identified by the sponsor

Food additive name	Food type	Concentration range submitted by sponsor (mg/kg)			Ranges of theoretical maximum daily exposure (mg/kg bw per day)		
		Minimum	Standard	Maximum	Minimum	Standard	Maximum
Potassium aluminium silicate	Solid food	1 000	10 000	12 500	6	60	78
	Liquid food	200	3 000	5 000	10	150	250
	Total	—	—	—	16	210	328

sponsor stated that it sells Candurin® pigments worldwide to food companies and has no information on sales volumes in different countries.

The usage of potassium aluminium silicate–based pearlescent pigments in food products in many countries is directly connected to the regulatory status of the food colours titanium dioxide and iron oxide. Furthermore, the pigments cannot be used in all foods, as there are certain technical restrictions regarding the application of the pigments within the group of permitted food products (e.g. stability and transparency). Based on these factors, potassium aluminium silicate–based pearlescent pigments represent a small portion of the global food colour market (estimated by the sponsor to be less than 0.2% in 2009).

(b) Screening by the budget method

As noted above in section 3.3.1(a), the budget method is used to assess theoretical maximum daily dietary exposure. The method relies on assumptions regarding 1) the level of consumption of foods and of non-milk beverages, 2) the use level of the substance in foods and in non-milk beverages and 3) the proportion of foods and of non-milk beverages that may contain the substance. For a person with a body weight of 60 kg, the levels of consumption are assumed to be 6 litres of non-milk beverages and 3 kg of food per day. The level of the additive used in foods is assumed to be the highest maximum level of the additive reported in any representative category, respectively, for foods and for beverages for which usage data were provided. The proportion of solid foods and beverages that may contain the substance is, respectively, 12.5% and 50%.

Table 9 summarizes the theoretical maximum daily exposure to potassium aluminium silicate–based pearlescent pigments for which uses have been identified by the sponsor. Three scenarios were considered by the Committee, based on the minimum efficacious level, the standard usage level and the maximum level reported by the sponsor for the products in which potassium aluminium silicate–based pearlescent pigments may be used.

The Committee noted that when potassium aluminium silicate–based pearlescent pigments are used in solid and liquid foods, the theoretical maximum

daily exposure based on the budget method would give rounded estimates ranging from 20 mg/kg bw per day (when using the reported minimum efficacious use level) up to 330 mg/kg bw per day (when using the maximum proposed use level).

(c) National estimates of dietary exposure

(i) Europe

According to Directive 95/2/EC as amended by Directive 2003/114/EC (see Attachment 01 for a consolidated version of Directive 95/2/EC including the amendments according to Directive 2003/114/EC), potassium aluminium silicate is allowed for use as a carrier for E171 titanium dioxide and E172 iron oxides and hydroxides (maximum 90% potassium aluminium silicate relative to the pigment). Regulation (EC) 1333/2008 (Attachment 02) repeals Directive 95/2/EC. However, according to Articles 30 and 34, this directive will remain in force during a transitional period until the European Community list of food additives has been established. The food additives will be entered in the relevant Annexes of Regulation (EC) 1333/2008 after a review of their compliance with Article 6.

Exposure estimates for European consumers have been made using the uses in food of potassium aluminium silicate–based pearlescent pigments at maximum use levels of the resulting pigments of 0.5% in beverages up to 1.25% by weight in solid foods proposed by the sponsor with the use of summary statistics (average all population and 95th percentile consumers only) from available EU food consumption data and following the rules of calculation defined by the EFSA Panel on Food Additives and Nutrient Sources Added to Food.

For children (aged 1–14 years, weighing 16–54 kg), anticipated exposures have been estimated by the Committee, based on summary statistics (average all population and 95th percentile consumers only) from detailed individual food consumption data from 11 European countries (Belgium, Cyprus, Czech Republic, Finland, France, Germany, Greece, Italy, the Netherlands, Spain and Sweden) provided by the EXPOCHI ("Individual food consumption data and exposure assessment studies for children") consortium (Huybrechts et al., 2011). As the United Kingdom is not part of the EXPOCHI consortium, estimates for children (aged 1.5–4.5 years) in the United Kingdom were made with the use of detailed individual food consumption data (United Kingdom National Diet and Nutrition Survey, 1992–1993) available from the Union of European Soft Drinks Associations (UNESDA) report (Tennant, 2008).

As the United Kingdom population is considered to be one of the highest consumers of soft drinks in Europe, it was decided to select the United Kingdom population as representative of EU consumers for potassium aluminium silicate exposure estimates for adults from an earlier report provided by UNESDA (Tennant, 2008).

For children, the data from the EXPOCHI countries and the United Kingdom data were used to calculate the mean and high-level exposures to potassium aluminium silicate–based pearlescent pigments using proposed maximum use levels. High-level exposure (95th percentile consumers only) was based on the assumption that an individual might be a high-level consumer of one food category

and an average consumer of the others. This approach has been tested several times by the EFSA Panel on Food Additives and Nutrient Sources Added to Food in each re-evaluation of food colours, and the results were in agreement with the exposure figures obtained by computer analysis using raw individual food consumption data. Therefore, this approach was preferred for the calculations based on the maximum proposed use levels in order to avoid excessively conservative estimates.

When considering the proposed maximum use levels, the dietary exposure to potassium aluminium silicate–based pearlescent pigments in European children ranged from 10 to 116 mg/kg bw per day at the mean and from 40 to 323 mg/kg bw per day at the 95th/97.5th percentile. The main contributors to the total anticipated exposure to potassium aluminium silicate–based pearlescent pigments (>10% in all countries) were non-alcoholic flavoured drinks (20–70%) and fine bakery wares (13–79%). Confectionery accounted for more than 10% of exposure in two European countries (1–20%).

For the United Kingdom adult population, the mean estimated dietary exposure to potassium aluminium silicate–based pearlescent pigments was 28 mg/kg bw per day, and the estimated dietary exposure for high-level consumers (97.5th percentile) was 86 mg/kg bw per day. The main contributors to the total anticipated exposure to potassium aluminium silicate–based pearlescent pigments (>10%) were non-alcoholic flavoured drinks (74%) and fine bakery wares (16%).

(ii) United States of America

The United States Food and Drug Administration (USFDA) performed an estimate of daily exposure to potassium aluminium silicate–based pearlescent pigments consisting of potassium aluminium silicate coated with titanium dioxide for consumers aged 2 years or more and children 2–5 years of age (USDA, 2006b). This estimate incorporated the maximum permitted use level of the potassium aluminium silicate–based pearlescent pigment in food of 1.25% by weight. The estimate included the categories of food for which titanium-containing potassium aluminium silicate–based pearlescent pigments are permitted for use as colour additives in the USA: cereals, confections and frostings, gelatine desserts, hard and soft candies (including lozenges), nutritional supplement tablets and gelatine capsules, and chewing gum. The Committee noted that beverages were not included as a proposed use by the sponsor when the USFDA made its evaluation.

Estimates of dietary exposure for consumers aged 2 years or more were 0.43 g/person per day at the mean and 0.86 g/person per day at the 90th percentile. Estimates of dietary exposure for children 2–5 years of age were 0.38 g/person per day at the mean up to 0.76 g/person per day at the 90th percentile (USDA, 2006b). In these estimates, gelatine candies and desserts prepared from gelatine powders and breakfast cereals were the major sources of the dietary exposures to the pigments (USFDA, 2005).

(iii) Conclusion

The Committee concluded that anticipated dietary exposure in the general population from the use of potassium aluminium silicate–based pearlescent

Table 10. Summary of anticipated exposure to potassium aluminium silicate–based pearlescent pigments in children and adult populations

	Exposure (mg/kg bw per day)			
	All (2+ years)* (USA)[a]	Children (2–5 years)* (USA)[a]	Adult (18+ years)*** (EU)	Children (1–14 years)**, *** (EU)
Mean exposure[b]	35	135	28	10–116
Exposure at the 90th*, 95th** or 97.5th*** percentile[b]	70	270	86	40–323

[a] For the USA population, estimates are based on assuming a 60 kg body weight for the general population and a 15 kg body weight for toddlers. At the time of the USFDA evaluation, beverages were not included in those estimates; the Committee provided an estimate for the USA population taking into account an average consumption of beverages of 330 ml (one can) per day.

[b] Assuming all processed foods and beverages contain colour added at maximum proposed use levels. Maximum use level in products coloured with titanium dioxide/iron dioxide pearl effect colours based on potassium aluminium silicate as the carrier of 0.5% in beverages up to 1.25% by weight in solid food.

pigments at the maximum proposed use levels (0.5% in beverages and 1.25% by weight in solid food) would range from 10 mg/kg bw per day at the mean to 323 mg/kg bw per day for consumers with a high dietary exposure. The Committee noted that in these conservative estimates, non-alcoholic flavoured drinks are the major contributor, from 20% up to 70%, to overall dietary exposure.

The estimates presented in Table 10 are for exposure to the potassium aluminium silicate–based pearlescent pigments themselves. In order to consider the contribution of the potassium aluminium silicate–based pearlescent pigments to aluminium exposure, the exposure values must first be converted to an aluminium basis. Taking into account a maximum level of 90% potassium aluminium silicate in the potassium aluminium silicate–based pearlescent pigments and the fact that potassium aluminium silicate is composed of 20% aluminium by weight (potassium aluminium silicate, $KAl_2[AlSi_3O_{10}](OH)_2$; relative molecular mass of 398), this corresponds to an aluminium exposure from potassium aluminium silicate–based pearlescent pigments of 1.8 mg/kg bw per day up to 58 mg/kg bw per day.

The Committee recognizes that its estimates could be considered as being conservative, as it is assumed that all processed foods and beverages contain the colour added at the maximum proposed use levels.

(d) International estimates of dietary exposure

The Committee considered it inappropriate to use the GEMS/Food consumption cluster diets to calculate international estimates of dietary exposure because potassium aluminium silicate is present mainly in prepared foods and not in staple foods.

4. COMMENTS

4.1 Toxicological data

As recommended by the Committee at its sixty-seventh meeting, new studies had been conducted on the bioavailability of aluminium compounds. The new data indicated that absorption of aluminium following the ingestion of various aluminium compounds by rats is generally in the region of 0.01–0.3% and support the assumption that the more water-soluble aluminium compounds are generally more bioavailable. As a result of limitations in the sensitivity of the analytical methods, inter-animal variation and methodological differences between studies, including the administered doses, it is not possible to draw firm conclusions on quantitative differences in absorption between different compounds. There are indications that there are sex differences in absorption in rats and that the proportion of the dose absorbed is lower following repeated administration than following single administration. The reported absorptions of the food additives for which data were available (sodium aluminium phosphate acidic, sodium aluminium phosphate basic, sodium aluminosilicate, aluminium sulfate, FD&C aluminium lake, aluminium metal, aluminium ammonium sulfate) are within the overall range of 0.01–0.3% in rats. A possible exception relates to potassium aluminium silicate–based pearlescent pigments. These products are marketed in particulate form. The solubility of the particulates is very low, and therefore it is likely that the bioavailability is lower than for other aluminium-containing food additives. However, direct data to support a conclusion that aluminium is appreciably less available from these pigments than from other aluminium compounds were not available.

In studies reviewed previously by the Committee, absorption of aluminium in human volunteers was within the same range as that in rats, with some indication of increased absorption in the elderly. The absorption can be modified by substances in foods that bind to the aluminium ion, such as citrate, which increases absorption, and phosphate, which forms an insoluble aluminium salt, thereby decreasing absorption. The newly available data indicate that absorption in humans is likely to vary widely, but did not support an estimation of bioavailability.

New studies in rats have confirmed that absorbed aluminium is able to cross the placental barrier into the fetus and then into the fetal brain and that it is also transferred to the offspring via lactation. The new studies have also confirmed that administration of a number of aluminium salts to rats can result in increased concentrations of aluminium in bone, kidney and spinal cord. About 90% of Al^{3+} in plasma is bound to transferrin, and about 10% to citrate. Cellular uptake is thought to occur from the aluminium bound to transferrin by transferrin receptor–mediated endocytosis.

No new data on excretion were identified. Studies reviewed previously by the Committee have shown that urine is the primary route of excretion of absorbed aluminium in experimental animals and in humans. Initial half-lives of 2–5 hours have been reported in rats, mice, rabbits and dogs after intravenous administration and less than 1 day in humans after intravenous administration. In different studies and species, multiple half-lives have been reported, arising from slower rates of elimination from different tissues.

Based on the available data relating to the absorption, distribution and elimination of aluminium from a variety of different aluminium compounds, the Committee concluded that there was no basis for deriving a chemical-specific adjustment factor for either interspecies or intraspecies differences in toxicokinetics.

As recommended by the Committee at its sixty-seventh meeting, new multigeneration reproductive and developmental toxicity studies incorporating neurobehavioural end-points had been conducted.

The multigeneration reproductive studies conducted with aluminium sulfate and aluminium ammonium sulfate administered to rats in the drinking-water did not provide evidence of reproductive toxicity. The major developmental effects observed in both studies were delayed maturation of the female offspring, decreased body weight gain and changes in some organ weights. These effects are likely to have been related to the reported decrease in maternal fluid and feed consumption. Thus, it is not possible to attribute the findings to a direct effect of the aluminium. No effects on motor activity or learning ability were observed in these studies.

The available developmental toxicity studies include two published studies involving dosing of aluminium chloride by oral gavage to pregnant rats. These studies provided evidence of fetotoxicity, but it was unclear if the findings were secondary to maternal toxicity. There were no effects on pregnancy outcome in a developmental study of aluminium chloride basic.

Cognitive deficits were observed in a number of new studies of neurotoxicity and neurobehavioural end-points. Most of these studies have limitations for use in risk assessment, such as administration of only one high dose level, failure to consider aluminium content in the diet, lack of assessment of other forms of toxicity and assessment of only a limited number of outcomes. The lowest aluminium dose linked with cognitive effects was 0.5 mg/kg bw per day administered to rats as aluminium chloride in the drinking-water, which was reported to be associated with impaired memory in old rats. In this study, the rats were given a restricted amount of feed twice weekly in order to reduce the rats' weight to approximately 85% of the free-feeding weight and hence prolong their lifespan. Typically, they ate the feed in the first 2–3 days and had a day or more with no feed. Whereas impaired cognitive function in old age is a potentially relevant observation, the impact of the restricted feeding regimen used in this study is unknown, and impaired cognitive function has been observed in other studies only at much higher levels of exposure, albeit in younger animals. The Committee therefore concluded that the results of this study require independent verification and were not suitable for use in the risk assessment.

In a developmental and chronic neurotoxicity study of aluminium citrate administered to rats in drinking-water, the major treatment-related effects were renal damage (hydronephrosis, urethral dilatation, obstruction and/or presence of calculi) and reduced grip strength, but not cognitive impairment, in the pups. Renal damage was not observed in a control group of rats given sodium citrate at the molar equivalent of the high-dose aluminium citrate, demonstrating that the effect was not due to the citrate ion. Dosing with both aluminium citrate and sodium citrate resulted in a significant increase in fluid consumption compared with control

animals. The NOAEL and LOAEL for these effects were at target aluminium doses of 30 and 100 mg/kg bw per day. However, because the aluminium citrate was administered in the drinking-water, the actual dose was influenced by the water consumption, which varied in the different stages of the study. Mean doses at the NOAEL were 10–14% below target during gestation, up to 50% above target during lactation, up to about 30% above target in the weaned pups for the first few weeks, but then 15–45% of target for the remainder of the study. At the LOAEL, the mean dosage level was approximately at target during gestation, up to 90% above target during lactation and the first few weeks post-weaning, and then 25–50% of target for the remainder of the study. Hence, if the effects in the pups were mediated in utero, the NOAEL is slightly overestimated; conversely, however, if the effects were mediated during lactation or the first few weeks after weaning, the NOAEL is underestimated. As the effect on grip strength was more pronounced in younger animals, exposure in utero and/or during lactation is likely to be more important than exposure during the later stages, when exposure was decreased due to decreased fluid consumption. The Committee concluded that, taking into account the greater bioavailability of aluminium from aluminium citrate than from other aluminium compounds, it was appropriate to assume that the NOAEL was 30 mg/kg bw per day. In view of the uncertainty regarding the doses at different times of this study as a result of changes in water consumption, the Committee decided not to model the dose–response data.

The Committee received a submission specifically on potassium aluminium silicate–based pearlescent pigments. No effects were observed in subchronic or chronic toxicity studies at doses of the test material up to 2500 mg/kg bw per day, equivalent to 360 mg/kg bw per day as aluminium, but no studies were available regarding reproductive or neurobehavioural effects.

Most epidemiological studies reviewed addressed the potential neurotoxicity of aluminium in drinking-water or antacids, by means of different designs: experimental, prospective cohort or case–control studies or ecological studies. The results of these studies were controversial; some of the drinking-water studies showed an association of aluminium with dementia or Alzheimer disease, whereas others reported an absence of neuropsychological effects measured in several ways. None of these studies took into account the ingestion of aluminium in food. The coincidental observation of neuropathological features of Alzheimer disease and aluminium in brain reported in some cases does not demonstrate a causal role of aluminium in Alzheimer disease. Occupational exposure to aluminium does not seem to have an impact on cognitive performance, motor performance or adverse reproductive outcomes in exposed workers. Although recent studies do not definitively rule out a positive association between aluminium in drinking-water and Alzheimer disease, the information available remains inconsistent and does not support a causal association. Neonates who were exposed to aluminium from solutions for parenteral nutrition had reduced lumbar spine and hip bone mass in adolescence. However, in elderly people, the aluminium content in bones was not associated with increased risk of hip fractures. There was no information from the epidemiological literature about the potential effects of oral exposure to aluminium in food. Given these limitations, no pivotal epidemiological studies are available for risk assessment.

4.2 Assessment of dietary exposure

Owing to their multiple functions, aluminium-containing food additives are permitted for use in a large variety of foods. At its present meeting, the Committee was asked to evaluate the safety of potassium aluminium silicate–based pearlescent pigments based on the recommendation of the Forty-second Session of CCFA (FAO/WHO, 2010). This aluminium-containing food additive has not previously been evaluated by the Committee.

Potassium aluminium silicate (mica) is used as a carrier substrate for titanium dioxide and/or iron oxide. Potassium aluminium silicate is not intended to be placed on the market as such, but only when coated with the food colours titanium dioxide and/or iron oxide. In the EU, E555 potassium aluminium silicate is approved as a carrier for E171 titanium dioxide and E172 iron oxides and hydroxides (maximum 90% potassium aluminium silicate relative to the pigment) (Directive 95/2/EC as amended by Directive 2003/114/EC). In the USA, pearlescent pigments consisting of potassium aluminium silicate coated with titanium dioxide are approved for use as a colour additive at levels up to 1.25% in cereals, confections and frostings, gelatine desserts, hard and soft candies (including lozenges), nutritional supplement tablets and capsules, and chewing gum (USFDA, 2006a). Potassium aluminium silicate–based pearlescent pigments are proposed to be used in confectionery, chewing gums and beverages at usage levels ranging from a minimum of 0.02% up to a maximum of 1.25%.

The Committee noted that no actual usage data were submitted for aluminium ammonium sulfate (INS 523), sodium aluminium phosphate basic (541(ii)), aluminium silicate (INS 559), aluminium powder or aluminium potassium sulfate (INS 522). Currently used aluminium-containing food additives are aluminium sulfate (INS 520), sodium aluminosilicate (INS 554), sodium aluminium phosphate acidic (INS 541(i)) and aluminium lakes of food colour.

At the sixty-seventh meeting, the Committee considered only consumer exposure to aluminium in the diet; occupational exposure and other routes or commodities were not considered. Dietary sources of exposure include natural dietary sources, drinking-water, migration from food contact materials and food additives. The potential range of exposure to aluminium from dietary sources reviewed at the sixty-seventh meeting by the Committee was 14–280 mg/week (see Table 8 in section 3.3.1).

For the evaluation of potassium aluminium silicate–based pearlescent pigments as a new food additive, the Committee evaluated an anticipated dietary exposure assessment based on food consumption data from the EU and the USA with the maximum proposed levels of use of potassium aluminium silicate–based pearlescent pigments. The Committee concluded that anticipated dietary exposure in the general population from the use of this food colour at the maximum proposed use levels (0.5% in beverages and 1.25% by weight in solid food) would range from 10 mg/kg bw per day at the mean to 323 mg/kg bw per day for consumers with a high consumption of non-alcoholic beverages. When converted to an aluminium basis, this corresponds to an aluminium exposure from potassium aluminium silicate–based pearlescent pigments of 1.8 mg/kg bw per day up to 58 mg/kg bw per day.

The Committee recognizes that its estimates are conservative, as it is assumed that all processed foods and beverages contain the colour added at the maximum proposed use levels. The Committee noted that non-alcoholic flavoured drinks are the major contributor in these estimates, accounting for 20–70% of overall dietary exposure.

For other aluminium-containing food additives under re-evaluation, a tentative estimate of dietary exposure from food additive sources has been made, taking into account previous assessments and other publications or submissions reviewed by the Committee at the current meeting. The Committee noted, from the report of its sixty-seventh meeting and from an EFSA scientific opinion, that the range of estimates was mainly based on dietary exposure calculated with the total diet study method, which takes into account water consumption. It is known from the literature that the main sources of migration of aluminium into food are from the use of cookware or aluminium utensils. It is also known that the design of total diet studies generally tries to control any bias of additional contamination that may result from the use of containers, cookware or utensils containing aluminium during the preparation and storage of food as consumed.

The Committee noted that estimates of the contribution to overall mean dietary exposure from all sources (including natural sources, water consumption, food contact materials and food additives) were in the range of 10–140 mg/week in adult populations (0.2–2.3 mg/kg bw per week as aluminium, assuming a body weight of 60 kg; see Table 8 in section 3.3.1 above). Major contributors to these estimates were cereals and cereal-based food products, with a proportion of 20–90%, depending on the country, equivalent to a dietary exposure of approximately 2–120 mg/week (0.03–2 mg/kg bw per week as aluminium, assuming a body weight of 60 kg).

This assessment is consistent with previous evaluations made by the Committee in which cereal products were considered as potentially high contributors to dietary aluminium exposure. The Committee also noted from its review that high levels of the actual uses of aluminium-containing food additives were reported for cereals and cereal-based products, in particular for sodium aluminosilicate (INS 554) and sodium aluminium phosphate acidic (INS 541(i)). Based on this, the Committee concluded that aluminium from the consumption of cereals and cereal-based products could reasonably be assumed to be mainly from food additive sources.

The Committee noted that the estimated dietary exposures related to average adult populations and that high dietary exposures (e.g. 90th or 95th percentile) are generally assumed to be 2 times higher than the reported average. It also noted that children generally have higher food intake than adults when expressed on a body weight basis and therefore represent the highest potential exposure to aluminium per kilogram of body weight.

5. EVALUATION

The new data submitted to the Committee and available in the published literature addressed some of the research needs identified previously, including

studies of bioavailability and reproductive, developmental and neurobehavioural effects.

The absorption of aluminium compounds is generally in the region of 0.01–0.3%. Soluble aluminium compounds appear to be more bioavailable, but it is not possible to draw conclusions on quantitative differences in the overall toxicokinetics of different aluminium-containing food additives or between experimental animals and humans.

The recent evidence did not show effects of aluminium on reproductive outcomes. The new studies support previous observations of neurodevelopmental effects in experimental animals, but there continues to be a lack of consistency regarding the reported effects, and there are some limitations to all of the studies. Most of the studies involved administration of aluminium compounds in drinking-water, rather than in the diet.

At its current meeting, the Committee noted that the new data did not substantially change the LOAEL range of 50–75 mg/kg bw per day, but one of the studies also provided a NOAEL of 30 mg/kg bw per day. This NOAEL was identified from a study in which aluminium citrate was administered in drinking-water. Aluminium citrate is more soluble than many other aluminium compounds and is likely to be more bioavailable from drinking-water than from food. The Committee concluded that the NOAEL of 30 mg/kg bw per day was an appropriate basis for establishing a PTWI for aluminium compounds. Because long-term studies on the relevant toxicological end-points had become available since the sixty-seventh meeting, there was no longer a requirement for an additional safety factor for deficiencies in the database. The Committee therefore established a PTWI of 2 mg/kg bw from the NOAEL of 30 mg/kg bw per day by applying a safety factor of 100 for interspecies and intraspecies differences. The previous PTWI of 1 mg/kg bw was withdrawn.

The data submitted on aluminium lactate and potassium aluminium silicate–based pearlescent pigments were insufficient to demonstrate that these food additives differ from other forms of aluminium in their bioavailability or toxicity. The PTWI applies to all aluminium compounds in food, including food additives. The Committee emphasized that whereas substances that have long half-lives and accumulate in the body are not generally considered suitable for use as food additives, consumption of aluminium-containing food additives would not be a health concern, provided that total dietary exposure to aluminium is below the PTWI.

The Committee concluded that, for adults, the estimates of mean dietary exposure to aluminium-containing food additives from consumption of cereals and cereal-based products are up to the PTWI of 2 mg/kg bw. Estimates of dietary exposure of children to aluminium-containing food additives, including high-level dietary exposure, can exceed the PTWI by up to 2-fold.

For potassium aluminium silicate–based pearlescent pigments at the maximum proposed use levels and using conservative estimates, the Committee noted that anticipated dietary exposure at the highest range of estimates is 200 times higher than the PTWI of 2 mg/kg bw.

Therefore, the Committee recommended that provisions for food additives containing aluminium included in the GSFA should be compatible with the revised PTWI for aluminium compounds of 2 mg/kg bw as aluminium from all sources.

There is a need for convincing data to demonstrate that aluminium is not bioavailable from potassium aluminium silicate–based pearlescent pigments.

No data were available to identify the forms of aluminium present in soya-based formula and their bioavailability. Such studies were requested at the sixty-seventh meeting and are still required.

6. REFERENCES

Abd-Elghaffar SKH, El Sokkary GH, Sharkawy AA (2007). Aluminum-induced neurotoxicity and oxidative damage in rabbits: protective effect of melatonin. *Biogenic Amines*, 21(4):225–240.

Ali MA, Vostacolaee E, Rahim C (2008). Effect of oral aluminum chloride administration during lactation on short and long-term memory of their offspring. *Journal of Biological Sciences*, 8(4):767–772.

Aparecida D (2009). *Exposicao a aditivos alimentares contend aluminio como fator de risco a saude publica non Brasil, Diretoria regional de Brasilia, Fundacao oswaldo cruz, 2009.* Submitted to FAO/WHO on 13 January 2011 by the Brazilian Ministry of Health, General Office of Food.

Aung NN, Yoshinaga J, Takahashi I (2006). Dietary intake of toxic and essential trace elements by the children and parents living in Tokyo Metropolitan Area, Japan. *Food Additives and Contaminants*, 23(9):883–894.

Azzaoui FZ, Ahami AO, Khadmaoui A (2008). Impact of aluminum sub-chronic toxicity on body weight and recognition memory of Wistar rat. *Pakistan Journal of Biological Sciences*, 11(14):1830–1834.

Bakar C et al. (2010). Effect of high aluminium concentration in water resources on human health, case study: Biga Peninsula, northwest part of Turkey. *Archives of Environmental Contamination and Toxicology*, 58:935–944.

Bataineh HN, Bataineh ZM, Daradka H (2007). Short-term exposure of female rats to industrial metal salts: effect on implantation and pregnancy. *Reproductive Medicine and Biology*, 6(3):179–183.

Beekhuijzen MEW (2007). *A combined 28-day repeated dose toxicity study with the reproduction/developmental toxicity screening test of aluminium chloride basic in rats by oral gavage.* 's-Hertogenbosch, the Netherlands, Notox B.V. (Notox Project 446941). Submitted to FAO/WHO by the International Aluminium Institute.

Bernard BK et al. (1990). Toxicology and carcinogenesis studies of dietary titanium dioxide–coated mica in male and female Fischer 344 rats. *Journal of Toxicology and Environmental Health*, 29:417–429.

Boom N (2008). *Aluminum in drinking water and Alzheimer's disease: analysis of the Canadian Study of Health and Aging prospective cohort* [MSc Thesis]. Ottawa, Ontario, Canada, University of Ottawa [cited in RSI, 2010].

Dziwenka M, Semple H (2009). *Bioavailability study for five aluminium salts in Sprague-Dawley rats.* Vegreville, Alberta, Canada, Alberta Research Council Inc. (Study TEH-128; Report No. TOA02908.02.rpt). Submitted to FAO/WHO by the International Aluminium Institute.

EC (1998). *Report on methodologies for the monitoring of food additive intake across the European Union. Final report submitted by the Task Coordinator, 16 January 1998.*

Brussels, Belgium, European Commission, Directorate General III Industry, Reports of a Working Group on Scientific Cooperation on Questions Relating to Food, Task 4.2 (SCOOP/INT/REPORT/2).

EFSA (2008). Scientific Opinion of the Panel on Food Additives, Flavourings, Processing Aids and Food Contact Materials on a request from European Commission on safety of aluminium from dietary intake. *The EFSA [European Food Safety Authority] Journal*, 754:1–34.

Erazi H, Sansar W, Ahboucha S (2010). Aluminum affects glial system and behaviour of rats. *Comptes Rendus Biologies*, 333(1):23–27.

Esparza JL, Garcia T, Gomez M (2011). Role of deferoxamine on enzymatic stress markers in an animal model of Alzheimer's disease after chronic aluminum exposure. *Biological Trace Element Research*, 141(1–3):232–245.

Exley C, Esiri MM (2006). Severe cerebral congophilic angiopathy coincident with increased brain aluminium in a resident of Camelford, Cornwall, UK. *Journal of Neurology, Neurosurgery & Psychiatry*, 77:877–879.

FAO/WHO (2001). *Guidelines for the preparation of working papers on intake of food additives for the Joint FAO/WHO Expert Committee on Food Additives.* Rome, Italy, Food and Agriculture Organization of the United Nations; Geneva, Switzerland, World Health Organization (http://www.who.int/ipcs/food/jecfa/en/intake_guidelines.pdf).

FAO/WHO (2009). Dietary exposure assessment of chemicals in food. In: *Principles and methods for the risk assessment of chemicals in food.* Rome, Italy, Food and Agriculture Organization of the United Nations; Geneva, Switzerland, World Health Organization (Environmental Health Criteria 240).

FAO/WHO (2010). *Report of the Forty-second Session of the Codex Committee on Food Additives, Beijing, China, 15–19 March 2010.* Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (ALINORM 10/33/12, Appendix XI; http://www.codexalimentarius.net/download/report/737/al33_12e.pdf).

FAO/WHO (2011). *Report of the Forty-third Session of the Codex Committee on Food Additives, Xiamen, China, 14–18 March 2011.* Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (REP11/FA; http://www.codexalimentarius.net/download/report/759/REP11_FAe.pdf).

Fewtrell MS et al. (2009). Aluminium exposure from parenteral nutrition in preterm infants: bone health at 15-year follow-up. *Pediatrics*, 124:1372–1379.

FSANZ (2011). *Dietary exposure to aluminium compounds in food, including additives.* Food Standards Australia New Zealand, 9 February 2011.

Fujii S (2009). *Two-generation toxicity study of aluminium sulfate in rats.* Sapporo, Japan, Safety Research Institute for Chemical Compounds Co., Ltd (Study No. SR07181). Submitted to FAO/WHO by the Ministry of Health, Labour and Welfare, Japan.

Fujii S (2010). *Two-generation toxicity study of aluminium ammonium sulfate in rats.* Sapporo, Japan, Safety Research Institute for Chemical Compounds Co., Ltd (Study No. SR07180). Submitted to FAO/WHO by the Ministry of Health, Labour and Welfare, Japan.

García T et al. (2009). Evaluation of the protective role of melatonin on the behavioural effects of aluminum in a mouse model of Alzheimer's disease. *Toxicology*, 265(1–2):49–55.

González-Weller D et al. (2010). Dietary intake of aluminum in a Spanish population (Canary Islands). *Journal of Agricultural and Food Chemistry*, 58(19):10452–10457.

Guo CH et al. (2009). Alterations in trace elements and oxidative stress in uremic patients with dementia. *Biological Trace Element Research*, 131:13–24.

Hellmann J, Broschard T (2005). *Iriodin® Ti 100 Color RY K, Iriodin® Colibri Red-brown K: re-evaluation of clinical and necropsy findings, laboratory analyses and re-examination of histopathology of Study T 9051.* Merck KGaA (Study No. T16330). Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Hellström HO et al. (2005). The aluminium content of bone increases with age, but is not higher in hip fracture cases with and without dementia compared to controls. *Osteoporosis International*, 16:1982–1988.

Heusener A, Von Eberstein M (1988). *EMD Iriodin® 502 C 63—Acute toxicity study in rats after oral administration.* Merck KGaA (Internal Study No. T13180). Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Hirata-Koizumi M et al. (2011a). Two-generation reproductive toxicity study of aluminium sulfate in rats. *Reproductive Toxicology*, 31(2):219–230.

Hirata-Koizumi M et al. (2011b). Evaluation of the reproductive and developmental toxicity of aluminium ammonium sulfate in a two-generation study in rats. *Food and Chemical Toxicology*, 49(9):1948–1959.

Huybrechts I et al. (2011). Dietary exposure assessments for children in Europe (the EXPOCHI project): rationale, methods and design. *Archives of Public Health*, 69:4.

IARC (1984). *Polynuclear aromatic compounds, Part 3. Industrial exposures in aluminium production, coal gasification, coke production, and iron and steel founding.* Lyon, France, International Agency for Research on Cancer (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, Volume 34).

ICGMA (2010). Data submitted to FAO/WHO on current use levels for aluminium sulfate (INS 520), sodium aluminosilicate (INS 554), sodium aluminium phosphate-acidic (INS 541i) and aluminium lakes of color by the International Council of Grocery Manufacturer Associations, 30 November 2010.

Itoh M et al. (2008). Progressive leukoencephalopathy associated with aluminium deposits in myelin sheath. *Journal of Child Neurology*, 23:938–943.

Jochmann G (1972). *Trial for subchronic toxicity in the 3-month feed test in rats.* Merck KGaA (Internal Study No. 4/146/72). Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Kieser H (1982). *Iriodin® Ti 100 Color RY K, Iriodin® Colibri Red-brown K, Iriodin® Colibri Blue-green K, Iriodin® Colibri Dark Blue K: Investigation of subchronic toxicity in rats in a 3-month feeding study with a 2-month treatment-free follow-up phase.* Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Kiesswetter E et al. (2007). Longitudinal study on potential neurotoxic effects of aluminium: I. Assessment of exposure and neurobehavioral performance of Al welders in the train and truck construction industry over 4 years. *International Archives of Occupational and Environmental Health*, 81:41–67.

Kiesswetter E et al. (2009). Longitudinal study on potential neurotoxic effects of aluminium: II. Assessment of exposure and neurobehavioral performance of Al welders in the automobile industry over 4 years. *International Archives of Occupational and Environmental Health*, 82:1191–1210.

Kramer PJ, Broschard T (2000a). Study No. T14771, revised version in English, including technical and scientific amendment from: Jochmann G (1972). *Trial for subchronic toxicity in the 3-month feed test in rats.* Merck KGaA (Internal Study No. 4/146/72). Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Kramer PJ, Broschard T (2000b). Internal Study No. T 9051, revised version in English, including technical and scientific amendment from: Kieser H (1982). *Iriodin® Ti 100 Color RY K, Iriodin® Colibri Red-brown K, Iriodin® Colibri Blue-green K, Iriodin® Colibri Dark Blue K: Investigation of subchronic toxicity in rats in a 3-month feeding study with a 2-month treatment-free follow-up phase.* Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Kumar A, Dogra S, Prakash A (2009). Protective effect of curcumin (*Curcuma longa*), against aluminium toxicity: possible behavioral and biochemical alterations in rats. *Behavioural Brain Research*, 205(2):384–390.

Mameli O et al. (2006). Effect of aluminum consumption on the vestibulo-ocular reflex. *Metabolic Brain Disease*, 21(2–3):89–107.

Merck (2010). Toxicological data for the evaluation of mica (potassium aluminium silicate) by JECFA. Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Meyer-Baron M et al. (2007). Occupational aluminium exposure: evidence in support of its neurobehavioral impact. *Neurotoxicology*, 28:1068–1078.

Molloy DW et al. (2007). Effects of acute exposure to aluminum on cognition in humans. *Journal of Toxicology and Environmental Health, Part A*, 70(23):2011–2019.

Pence DH, Osheroff MR (1987). *Combined oral chronic toxicity and oncogenicity study in rats with titanium dioxide coated mica.* Hazleton Laboratories America on behalf of Merck KGaA (Study No. 2164-100). Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Poirier J et al. (2011). Double-blind, vehicle-controlled randomized twelve month neurodevelopmental toxicity study of common aluminum salts in the rat. *Neuroscience*, 193:338–362.

Prakash A, Kumar A (2009). Effect of *N*-acetyl cysteine against aluminium-induced cognitive dysfunction and oxidative damage in rats. *Basic Clinical Pharmacology and Toxicology*, 105(2):98–104.

Priest N (2010). *The bioavailability of ingested Al-26 labelled aluminium and aluminium compounds in the rat*. Mississauga, Ontario, Canada, Atomic Energy of Canada Ltd (unpublished General Nuclear Product Report GNP-121100-REPT-001). Submitted to FAO/WHO by the International Food Additives Council.

Ribes D et al. (2008). Effects of oral aluminum exposure on behavior and neurogenesis in a transgenic mouse model of Alzheimer's disease. *Experimental Neurology*, 214(2):293–300.

Roig JL et al. (2006). Aluminum, restraint stress and aging: behavioural effects in rats after 1 and 2 years of aluminum exposure. *Toxicology*, 218(2–3):112–124.

Rondeau V et al. (2006). Analysis of the effect of aluminium in drinking water and transferrin C2 allele on Alzheimer's disease. *European Journal of Neurology*, 13:1022–1025.

Rondeau V et al. (2009). Aluminium and silica in drinking water and the risk of Alzheimer's disease or cognitive decline: findings from 15-year follow-up of the PAQUID cohort. *American Journal of Epidemiology*, 169:489–496.

Rose M et al. (2010). Dietary exposure to metals and other elements in the 2006 UK Total Diet Study and some trends over the last 30 years. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Exposure, Control & Risk Assessment*, 27(10):1380–1404.

RSI (2010). *Developmental and neurological effects associated with oral exposure to aluminium-containing substances: a review of recent scientific evidence (2006 to present).* Ottawa, Ontario, Canada, Risk Sciences International. Submitted to FAO/WHO by the International Aluminium Institute.

Saiyed SM, Yokel RA (2005). Aluminium content of some foods and food products in the USA, with aluminium food additives. *Food Additives and Contaminants*, 22(3):234–244.

Sakr CJ et al. (2010). Reproductive outcomes among male and female workers at an aluminium smelter. *Journal of Occupational and Environmental Medicine*, 52:137–143.

Semple H (2010). *One-year developmental and chronic neurotoxicity study of aluminium citrate in rats.* Vegreville, Alberta, Canada, Alberta Research Council Inc. (Study TEH-113; Report No. TOA02982.03.rpt). Submitted to FAO/WHO by the International Aluminium Institute.

Sethi P et al. (2008). Aluminium-induced electrophysiological, biochemical and cognitive modifications in the hippocampus of aging rats. *Neurotoxicology*, 29(6):1069–1079.

Sethi P et al. (2009). Curcumin attenuates aluminium-induced functional neurotoxicity in rats. *Pharmacology, Biochemistry and Behavior*, 93(1):31–39.

Sharma P, Mishra KP (2006). Aluminum-induced maternal and developmental toxicity and oxidative stress in rat brain: response to combined administration of Tiron and glutathione. *Reproductive Toxicology*, 21(1):313–321.

St Laurent J (2006). *Solubility of 6 pigment products in simulated gastric and intestinal juices monitored using aluminium and iron analysis by atomic absorption.* Chemic Laboratories, Inc. on behalf of EMD Chemicals (Report No. 06-1706, Version 2; Laboratory Project ID 3261A). Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Sunaga M (2010a). *Single dose bioavailability study of aluminium ammonium sulfate in rats.* Sapporo, Japan, Safety Research Institute for Chemical Compounds Co., Ltd (Study No. SR07178). Submitted to FAO/WHO by the Ministry of Health, Labour and Welfare, Japan.

Sunaga M (2010b). *Repeated dose bioavailability study of aluminium ammonium sulfate in rats.* Sapporo, Japan, Safety Research Institute for Chemical Compounds Co., Ltd (Study No. SR07179). Submitted to FAO/WHO by the Ministry of Health, Labour and Welfare, Japan.

Sunaga M (2010c). *Single dose bioavailability study of aluminium lactate in rats.* Sapporo, Japan, Safety Research Institute for Chemical Compounds Co., Ltd (Study No. SR07176). Submitted to FAO/WHO by the Ministry of Health, Labour and Welfare, Japan.

Sunaga M (2010d). *Repeated dose bioavailability study of aluminium lactate in rats.* Sapporo, Japan, Safety Research Institute for Chemical Compounds Co., Ltd (Study No. SR07177). Submitted to FAO/WHO by the Ministry of Health, Labour and Welfare, Japan.

Sunaga M (2010e). *Single dose bioavailability study of aluminium sulfate in rats.* Sapporo, Japan, Safety Research Institute for Chemical Compounds Co., Ltd (Study No. SR07174). Submitted to FAO/WHO by the Ministry of Health, Labour and Welfare, Japan.

Sunaga M (2010f). *Repeated dose bioavailability study of aluminium sulfate in rats.* Sapporo, Japan, Safety Research Institute for Chemical Compounds Co., Ltd (Study No. SR07175). Submitted to FAO/WHO by the Ministry of Health, Labour and Welfare, Japan.

Tennant DR (2008). Screening potential intakes of colour additives used in non-alcoholic beverages. *Food and Chemical Toxicology*, 46:1985–1993.

USFDA (2005). Memorandum from Lee, Chemistry Review Group, Division of Petition Review, to DeLeo, Regulatory Group II, Division of Petition Review, March 1, 2005 [cited in USFDA, 2006b].

USFDA (2006a). *Mica-based pearlescent pigments.* Silver Spring, MD, USA, Department of Health and Human Services, Food and Drug Administration (21 CFR Part 73, section 73.350, 25 May 2006; http://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfcfr/CFRSearch.cfm?fr=73.350).

USFDA (2006b). Listing of color additives exempt from certification; mica-based pearlescent pigments. Final rule. *Federal Register*, 71(106):31927–31929 (http://www.gpo.gov/fdsys/pkg/FR-2006-06-02/pdf/E6-8575.pdf).

Utesch D (2000). *Art. 120608 Candurin® Honeygold—Micronucleus test in rats after oral administration.* Merck KGaA (Internal Study No. T14787). Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Utesch D (2006). *Mica pigment mix (Art. 104670, Art. 104634, Art. 104976, 1:1:1)—Bacterial mutagenicity assay,* Salmonella typhimurium *and* Escherichia coli. Merck KGaA (Internal Study No. T16346). Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Von Eberstein M (1971). *Iriodin® Ti 100 K—Trial for acute toxicity in dogs after oral administration.* Merck KGaA (Internal Study No. 4/37/71). Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Von Eberstein M (1975). *Nacreous Pigment Iriodin® Colibri Reddish-brown 04502 K—Trial for acute toxicity in rats after oral administration and for primary skin and mucosal irritation in rabbits.* Merck KGaA (Internal Study No. 4/95/75). Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Von Eberstein M, Rogulja P (1970). *Iriodin®: Trial for acute toxicity in rats after oral administration and for primary skin and mucosal irritation in rabbits.* Merck KGaA (Internal Study No. 4/76/70). Submitted to FAO/WHO by Merck KGaA, 19 November 2010.

Walton JR (2006). Aluminium in hippocampal neurons from humans with Alzheimer's disease. *Neurotoxicology*, 27:385–394.

Walton JR (2007). A longitudinal study of rats chronically exposed to aluminum at human dietary levels. *Neuroscience Letters*, 412(1):29–33.

Walton JR (2009). Functional impairment in aged rats chronically exposed to human range dietary aluminum equivalents. *Neurotoxicology*, 30(2):182–193.

Walton JR (2010). Evidence for participation of aluminium in neurofibrillary tangle formation and growth in Alzheimer's disease. *Journal of Alzheimer's Disease*, 22:65–72.

Wong WW et al. (2010). Dietary exposure to aluminium of the Hong Kong population. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 27(4):457–463.

Wu Y (2011). *Report for 2007 Chinese Total Diet Study.* Submitted to FAO/WHO by the Chinese Center for Disease Control and Prevention.

Yokel RA, Florence RL (2006). Aluminum bioavailability from the approved food additive leavening agent acidic sodium aluminum phosphate, incorporated into a baked good, is lower than from water. *Toxicology*, 227(1–2):86–93.

Yokel RA, Florence RL (2008). Aluminum bioavailability from tea infusion. *Food and Chemical Toxicology*, 46(12):3659–3663.

Yokel RA, Hicks CL, Florence RL (2008). Aluminum bioavailability from basic sodium aluminum phosphate, an approved food additive emulsifying agent, incorporated in cheese. *Food and Chemical Toxicology*, 46(6):2261–2266.

Yokel RA et al. (2001). Aluminum bioavailability from drinking water is very low and is not appreciably influenced by stomach contents or water hardness. *Toxicology*, 161(1–2):93–101.

Yumoto S et al. (2009). Demonstration of aluminium in amyloid fibers in the cores of senile plaques in the brains of patients with Alzheimer's disease. *Journal of Inorganic Biochemistry*, 103:1579–1584.

Zhou Y, Yokel RA (2006). The effect of citrate, maltolate and fluoride on oral ^{26}Al absorption. *Experimental Biology*, 2006: Abstract 710.5.

Zhou Y, Harris WR, Yokel RA (2008). The influence of citrate, maltolate and fluoride on the gastrointestinal absorption of aluminum at a drinking water relevant concentration: A ^{26}Al and ^{14}C study. *Journal of Inorganic Biochemistry*, 102(4):798–808.

BENZOE TONKINENSIS

First draft prepared by

R. Danam,[1] M. DiNovi[1] and J. Schlatter[2]

[1] Office of Food Additive Safety, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, Maryland, United States of America (USA)
[2] Nutritional and Toxicological Risks Section, Federal Department of Home Affairs, Federal Office of Public Health, Zurich, Switzerland

1. EXPLANATION

Benzoe Tonkinensis is a balsamic resin from the *Styrax tonkinensis* (Pierre) Craib ex Hartwich tree, which belongs to the Styracaceae family. It is variously referred to as Siam benzoin gum, Siam benzoin and benzoin Laos or in a generic way as "benzoin gum".

Two varieties of benzoin gums occur: Benzoe Tonkinensis and Benzoe Sumatranus. These two resins differ in their botanical source, geographical origin and chemical composition. The term "benzoin gum" can include resins from one or the other of the two sources or their mixtures.

The Committee previously considered benzoin gum at its twenty-first and fifty-fifth meetings (Annex 1, references *44* and *149*) but did not evaluate it owing to the lack of analytical and toxicological data. At its twenty-first meeting, the Committee prepared a tentative specification covering the two forms of benzoin gum. However, no acceptable daily intake (ADI) was established, and no monograph was prepared. At its fifty-fifth meeting, the Committee withdrew the tentative specification for benzoin gum, as the relevant information was not provided.

Benzoe Tonkinensis is intended to be used as a flavouring agent in foods and beverages.

1.1 Chemical and technical considerations

1.1.1 Sources and processing of Benzoe Tonkinensis and Benzoe Sumatranus

Benzoe Tonkinensis is a benzoin gum obtained by tapping the gum that exudes after a deep incision is made through the bark of the tree *Styrax tonkinensis* (Pierre) Craib ex Hartwich. The hardened gum is harvested, cleaned, sorted and graded according to the size of the pieces. Commercially, it is available in four different grades: 1, 2, 3 and 5. They are described as follows: Grade 1, very large tears, yellow-orange, without any foreign particles; Grade 2, large tears, orange-yellow; Grade 3, small tears, orange; Grade 5, very small and agglomerated tears, almost powder-like, deep red, contains a lot of foreign particles, such as pieces of wood and bark (Fernandez et al., 2003). Benzoe Tonkinensis is beige to brownish in colour, with a pleasant, sweet balsamic odour and a distinct note of vanilla. It is used in the flavour and fragrance industry, particularly in brown flavours such as vanilla, chocolate and nuts. It is produced mainly in the Lao People's Democratic Republic, Thailand and Viet Nam.

Benzoe Sumatranus is the second type of benzoin gum obtained from other *Styrax* species: *Styrax benzoin* Dryander and *Styrax parallelo neurum* Perkins. Benzoe Sumatranus (benzoin gum Sumatra) presents a strong styrax-like odour, quite distinct from the vanilla odour of the Siam variety. Benzoe Sumatranus is available in four grades: A, B, C and D. They are described as follows: Grade A, very large tears, yellow-brown, without any foreign particles; Grade B, large tears, brown-yellow; Grade C, small tears, brown; Grade D, very small and agglomerated tears, almost powder-like, dark brown–reddish, contains a lot of foreign particles, such as pieces of wood and bark (Fernandez et al., 2003). It is often used in soaps,

detergents, tobacco products and adhesives. It is produced mainly in Indonesia, especially Sumatra and Java.

1.1.2 Composition of Benzoe Tonkinensis

Benzoe Tonkinensis has an opaque appearance and consists of grainy, ovoid, flattened almond-like splits, sometimes agglomerated by a brown-red transparent resin. The product has a strong vanilla flavour and is insoluble in water and soluble in ethanol.

Benzoe Tonkinensis is a complex mixture of various chemical compounds. The resin is composed mainly of coniferyl benzoate (15–60%) and benzoic acid (15–45%), with lesser amounts of vanillin (<5%), benzyl benzoate (<2%), 2-hydroxy-1-phenylethanone and 1-(4-hydroxy-3-methoxyphenyl)-2-propanone. The Committee noted that there are large variations between samples of Benzoe Tonkinensis in the amounts of the four main components (coniferyl benzoate, benzoic acid, vanillin and benzyl benzoate) determined in the ethanolic extract. Further, the resin contains a significant amount of unidentified compounds in the ethanolic extract.

Extraction of the crushed gum with methanol:methylene chloride (50:50) in hot conditions for 3 hours yields resinoid composed of volatile, non-volatile and insoluble compounds. The components of the volatile fraction constitute 20–50% of the total resinoid. Fourteen volatile components were detected, of which 11 were identified. The five major compounds in the volatile fraction are benzoic acid (80%), vanillin (5%), benzyl benzoate (4%), 2-hydroxy-1-phenylethanone (2%) and 1-(4-hydroxy-3-methoxyphenyl)-2-propanone (1.5%). These compounds do not appear to vary considerably with production basin (place of harvest), year of harvest or even the grade of the gum. Unidentified compounds of the volatile fraction constitute about 2–7%. The composition of the non-volatile fraction that makes up about 50–80% of the resinoid has not yet been determined. This chemical composition of the gum is comparable to that found in other published literature (Fernandez et al., 2003; Castel et al., 2006a,b).

Nine triterpenoids—6β-hydroxy-3-oxo-11α,12α-epoxyolean-28,13β-olide (1), 3β,6β-dihydroxy-11α,12α-epoxyolean-28,13β-olide (2), 3β,6β-dihydroxy-11-oxo-olean-12-en-28-oic acid (3), 3β-hydroxy-12-oxo-13Hα-olean-28,19β-olide (4), 19α-hydroxy-3-oxo-olean-12-en-28-oic acid (5), 6β-hydroxy-3-oxo-olean-12-en-28-oic acid (6), sumaresinolic acid (7), siaresinolic acid (8) and oleanolic acid (9)—were isolated from the resin of *Styrax tonkinensis* extracted with 95% ethanol. The structures of these triterpenoids were determined by physicochemical and spectroscopic methods. However, no quantitative information was given (Wang et al., 2006).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

There are no data available on the absorption, distribution, metabolism or excretion of Benzoe Tonkinensis. There are also no data available on the effects of Benzoe Tonkinensis on enzymes and other biochemical parameters.

2.2 Toxicological studies

2.2.1 Previous evaluations of the major components of the volatile fraction of Benzoe Tonkinensis by the Committee

The flavouring agent benzoic acid (No. 850) was evaluated at the sixth, ninth, seventeenth, twenty-seventh and forty-sixth meetings of the Committee (Annex 1, references *6*, *11*, *32*, *62* and *122*). Benzyl benzoate (No. 24) was evaluated at the fifteenth and forty-sixth meetings of the Committee (Annex 1, references *26* and *122*). At its forty-sixth meeting, the Committee concluded that the flavouring agents in the group of benzyl derivatives, including benzoic acid and benzyl benzoate, would not present any safety concerns when used at the estimated current levels and confirmed the group ADI of 0–5 mg/kg body weight (bw) as benzoic acid equivalents (Annex 1, reference *122*). The Committee maintained this group ADI at its fifty-seventh meeting (Annex 1, reference *154*).

Vanillin (No. 889) was evaluated at the eleventh meeting of the Committee and assigned an ADI of 0–10 mg/kg bw (Annex 1, reference *14*). At its fifty-seventh meeting, the Committee concluded that there were no safety concerns at current levels of exposure when vanillin was used as a flavouring agent and maintained the ADI of 0–10 mg/kg bw (Annex 1, reference *154*).

2.2.2 Acute toxicity

There are no acute toxicity studies on Benzoe Tonkinensis.

The acute toxicity of benzoin gum was examined in rats and rabbits (Table 1). The Committee noted that the nature and source of the benzoin gum were not specified.

(a) Cell growth and differentiation assay

In an in vitro cell growth and differentiation assay using the human acute promyelocytic leukaemia cell line HL-60, all nine triterpenoids isolated from *Styrax tonkinensis* were tested for their effects on cell growth. The results of the study indicated that all nine triterpenoids inhibited HL-60 cell growth, with median growth inhibitory concentration (IG_{50}) values ranging from 8.9 to 99.4 µmol/l. Oleanolic acid (9) was the most effective antiproliferative agent, with an IG_{50} value of 8.9 µmol/l. Although 3β,6β-dihydroxy-11-oxo-olean-12-en-28-oic acid (3) exhibited the least effective growth inhibition among these triterpenoids, it induced HL-60 cells to undergo differentiation as measured by a nitroblue tetrazolium reduction assay. Cytotoxicity was observed only in cells treated with the compounds 3β,6β-dihydroxy-11α,12α-epoxyolean-28,13β-olide (2), 3β-hydroxy-12-oxo-13Hα-olean-28,19β-olide (4), sumaresinolic acid (7) and oleanolic acid (9) at concentrations twice the IG_{50} values (loss of more than 20% viable cells) (Wang et al., 2006).

2.2.3 Short-term studies of toxicity

In a 90-day oral toxicity study, Wistar rats of strain Crl:WI (10 of each sex per group) were administered Benzoe Tonkinensis once daily by gavage (using

Table 1. Acute toxicity of benzoin gum

Species	Route	LD_{50} (mg/kg bw)	References
Rat	Oral	10 000	Margolin (1970a); Opdyke (1973)
Rabbit	Dermal	8 870	Margolin (1970b); Opdyke (1973)

LD_{50}, median lethal dose

stainless steel ball–tipped oral intubation needle). The test substance Benzoe Tonkinensis (purity 97%) was suspended in corn oil and administered at a dose volume of 10 ml/kg bw. Test substance formulations were prepared fresh daily prior to the administration. Rats were administered Benzoe Tonkinensis in corn oil by gavage at dose levels of 0 (vehicle control), 500, 1000 or 2000 mg/kg bw per day; the study included two satellite groups of vehicle control and a high-dose group (2000 mg/kg bw per day) kept for an additional 28 days without any treatment to evaluate any possible reversible effects after withdrawal of the treatment. The Committee noted that the test material was inadequately characterized. The study was performed according to Organisation for Economic Co-operation and Development (OECD) Test Guideline 408 (1998) and certified for compliance with good laboratory practice (GLP). The animals were examined once daily throughout the study for clinical signs, and body weights were measured weekly. Cage-wise feed consumption was recorded daily and reported group-wise weekly throughout the study period. Test parameters for the study included morbidity and mortality, functional observational battery tests, ophthalmoscopic examination, haematology, clinical biochemistry, urinalysis, gross pathology and histopathology. Organ weights of liver, kidneys, adrenals, testes, epididymides, uterus, ovary, thymus, spleen, brain and heart were recorded. On day 91, all animals except those in the satellite groups were sacrificed. On day 119, animals from the satellite groups were sacrificed.

During the entire course of the study (both treatment and post-treatment periods), no clinical signs of toxicity or mortality were observed in animals from any of the groups. No statistically significant differences were observed in the body weights of animals in any group. In male rats, no statistically significant changes were observed in feed consumption in the low-, intermediate- and high-dose groups when compared with the control group. However, feed consumption in the satellite high-dose group showed a statistically significant increase at the 8th, 11th, 14th and 16th weeks when compared with the satellite vehicle control group. In females, a statistically significant increase in feed consumption was observed in the low- and high-dose groups at the 12th week when compared with the vehicle control group and in the satellite high-dose group at the 4th, 6th and 8th weeks when compared with the satellite vehicle control group. Although these observed changes are statistically significant, they were not dose dependent; hence, the authors concluded that they were not treatment related. Ophthalmological examination and functional observational battery parameters recorded prior to administration of the test substance and during the 13th and 17th weeks did not show any significant changes in any of the groups. There were no statistically significant treatment-related changes in haematological parameters in any group. In male rats, clinical biochemistry parameters analysed on day 91 showed statistically significant increases in alanine aminotransferase (ALT)

activity in the high-dose and satellite high-dose groups, whereas in female rats, also on day 91, both ALT activity and cholesterol levels increased in a dose-dependent manner in the intermediate-, high- and satellite high-dose groups compared with the control rats. On day 119, in both male and female rats, the changes in ALT activity and cholesterol level did not show any statistical significance, indicating that these changes were reversible after the termination of treatment on day 91. In both males and females, there were no statistically significant changes in the urinalysis parameters in any of the groups on days 1, 91 and 119. No treatment-related abnormalities in gross pathology were detected in any group.

In males, statistically significant increases in the relative organ weights of brain, liver, spleen, heart and kidneys were observed in the high-dose group when compared with vehicle controls. Similarly, in females, statistically significant increases in the absolute and relative weights of liver were observed in the high-dose group when compared with the vehicle control group. However, the satellite groups did not show any statistically significant changes in either sex in absolute or relative organ weights. Histopathological examination of both males and females revealed treatment-related lesions such as multinucleated cells in liver in the high-dose group, whereas bile duct hyperplasia and lacteal ectasia (prominent white villi) in the jejunum were observed in the intermediate- and high-dose groups, which persisted in the satellite high-dose group. The observations in the liver correlated with the observed organ weight changes in the high-dose group. The Committee concluded that these effects in the liver would not progress to neoplastic alterations. Based on these results, the authors concluded that the no-observed-adverse-effect level (NOAEL) was 500 mg/kg bw per day (Giridharrao et al., 2010).

2.2.4 Long-term studies of toxicity and carcinogenicity

Data are not available from long-term studies of toxicity and carcinogenicity with Benzoe Tonkinensis.

2.2.5 Genotoxicity

Three in vitro studies of genotoxicity with Benzoe Tonkinensis gave no indication of genotoxic activity. The results are summarized in Table 2. The Ames test was performed in accordance with OECD Test Guideline 471 (1997), the study on chromosomal aberrations was performed in accordance with OECD Test Guideline 473 (1997) and the mouse lymphoma assay was performed in accordance with OECD Test Guideline 476 (1997). All of the studies were certified for compliance with GLP and quality assurance. The Committee noted that there was cytotoxicity in both bacterial cells and human lymphocytes at relatively low concentrations. The observed cytotoxicity may be due to the presence of some triterpenoids, such as oleanolic acid, in the resin.

2.2.6 Reproductive and developmental toxicity

Data are not available on the reproductive toxicity of Benzoe Tonkinensis. However, neither benzoin gum nor the major constituents of its volatile fraction (i.e. benzoic acid, vanillin and benzyl benzoate) are teratogenic (Annex 1, references *122* and *154*).

Table 2. Results of in vitro genotoxicity assays with Benzoe Tonkinensis

Test system	Test object	Concentration	Results	Reference
Bacterial reverse mutation test[a]	*Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537 and *Escherichia coli* WP2uvrA	0.26, 0.78, 2.33, 7.00 and 21.00 µg/plate[b]	Negative	Escarti (2010a)
Chromosomal aberration assay[a]	Human lymphocytes, 4 h exposure and 32 h expression period	20, 40, 78.13, 80 and 156.25 µg/ml	Negative	Mohan (2010a)
Chromosomal aberration assay	Human lymphocytes, continuous exposure (without S9) and 36 h expression period	10, 20 and 40 µg/ml[c]	Negative	Mohan (2010a)
Gene mutation assay[a] (3 h study)	L5178Y TK+/– mouse lymphoma cell line	19.54, 39.04, 78.13 and 156.25 µg/ml[d]	Negative	Mohan (2010b)
Gene mutation assay[a] (24 h study)	L5178Y TK+/– mouse lymphoma cell line	9.77, 19.54, 39.04 and 78.13 µg/ml[d]	Negative	Mohan (2010b)

S9, 9000 × *g* rat liver supernatant

[a] Both with and without rat liver S9 fraction. Test repeated with pre-incubation method.

[b] Cytotoxic at test doses of 62–5000 µg/plate (Escarti, 2010b). Test repeated with pre-incubation method.

[c] Cytotoxic at concentrations of 78.13, 80 and 156.25 µg/ml.

[d] Cytotoxic at concentrations of 312.5–5000 µg/ml.

2.2.7 Special studies

(a) Allergic responses

(i) Guinea-pig sensitization test

Benzoin Siam oil, a perfume ingredient, was tested for its sensitization potential in albino guinea-pigs (inbred Hartley strain) by the modified Draize technique. Ten guinea-pigs were injected at four sites intradermally with 0.625% Benzoin Siam oil to induce sensitization and challenged 2 weeks later by intradermal injection of 0.25% Benzoin Siam oil and topical application of 10% Benzoin Siam oil. Twenty-four hours later, the reactions were scored. No reactions were seen at the challenge. The procedures of induction and challenge were repeated 7 days later on the same animals. At each challenge with controls, four previously untreated animals of the same sex and weight as the test animals were included. There was evidence of sensitization after two treatments with Benzoin Siam oil. The author suggested that Benzoin Siam oil showed a weak sensitization potential (Sharp, 1978). The Committee noted that it is unclear whether the composition of Benzoin Siam oil is identical to that of Benzoe Tonkinensis.

2.3 Observations in humans

2.3.1 Contact sensitization potential

There are no data available on the effects of Benzoe Tonkinensis in humans. However, there are several case reports in humans illustrating dermal contact sensitization with tincture of benzoin, dating back to 1874 (Fox, 1874). Benzoin gum has been used therapeutically as an essential ingredient in tincture of benzoin, which consists of 10% benzoin gum in alcohol (Coskey, 1978). However, the botanical source of the benzoin gum in the tincture is not specified in any of the case reports listed below.

An unusual benzoin hypersensitivity was reported in a 22-year-old student who developed acute eczematous contact dermatitis locally in response to the application of tincture of benzoin to the skin under a cast. Later, a non-eczematous exanthem developed within 48 hours. The authors concluded that absorption through the skin was responsible for both the contact dermatitis and the exanthem (Spott & Shelley, 1970).

Closed patch tests indicated that tincture of benzoin showed strong reactions: 45 out of 477 patients presented positive responses to benzoin tincture. Of these 45 patients, 14 had strong positive reactions to compound tincture of benzoin, and 11 had at least one other positive cross-reaction to similar allergens (fragrance mix, balsam of Peru, colophony and tea tree oil) (Scardamaglia, Nixon & Fewings, 2003).

In two large studies, out of a total of 1835 patients (probably with contact dermatitis), 35 individuals developed positive reactions to 10% benzoin gum in ethanol when patch tested, presumably for 24 or 48 hours under cover. It was reported that those sensitized apparently cross-react to balsam of Peru, storax, eugenol, vanillin, α-pinene, benzyl alcohol and benzyl cinnamate (BIBRA, 1989).

Benzoin caused sensitization clinically but did not sensitize when tested by the Kligman maximization test procedure (Opdyke, 1973).

Benzoin used as a glaze confectionery was reported to be a skin sensitizer (Fisher, 1982).

3. DIETARY EXPOSURE

3.1 Introduction

The Committee received one submission that contained information concerning food uses of and estimated dietary exposure to Benzoe Tonkinensis (Agroforex, 2011). Benzoe Tonkinensis can be used as a flavouring agent in both solid foods and beverages. The sponsor noted that it has been used in foods in Europe and has been designated as a generally recognized as safe flavouring agent by the Flavor and Extract Manufacturers Association of the United States.

3.2 International estimates of exposure

The Committee concluded that use of the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/

Food) consumption cluster diets was inappropriate for estimating dietary exposure to Benzoe Tonkinensis, as it is used exclusively as a minor ingredient in processed food products.

3.3 National estimates of exposure

3.3.1 Uses and use levels

The sponsor noted the following technical uses of Benzoe Tonkinensis in food: as a flavour, anticaking agent, coating agent, glazing agent, texturizing agent and preservative. Benzoe Tonkinensis also finds use in pharmaceuticals and cosmetics. However, these latter uses were not considered by the Committee in the dietary exposure assessment, as cosmetic uses are not relevant to dietary exposure and pharmaceutical uses would not likely contribute significantly to overall chronic dietary exposure.

The request to the Committee was to evaluate the use of Benzoe Tonkinensis as a flavouring agent in solid foods at levels up to 10 mg/kg and in beverages at levels up to 5 mg/kg.

The dossier contained information on current uses of Benzoe Tonkinensis around the world. In the USA, it is used in sweets, “exotic meals”, drinks with or without alcohol and dairy products (jellied or not). The incorporation rate varies, ranging between 150 mg/kg (for delicacies) and 40 000 mg/kg (for chocolate bars). In Japan, Benzoe Tonkinensis is used as a chewing gum base as well as in baked goods at concentrations up to 140 mg/kg and in frozen dairy products at concentrations up to 75 mg/kg. In Thailand, it is used in flavoured desserts, whereas in France, it was used in chewing gum until 1991 at quantum satis concentrations. Levels as high as 140 000 mg/kg in chewing gum have been reported in the literature. Although these uses are not consistent with the levels requested by the sponsor, the Committee considered exposure from all food sources as well as the requested flavouring agent use.

3.3.2 Budget method

The sponsor included a budget method to evaluate the theoretical maximum dietary exposure to Benzoe Tonkinensis. Using the assumptions that the maximum daily intakes of solid food and beverages are 1.5 kg and 6 litres, respectively, and that Benzoe Tonkinensis would be used in 100% of each at levels of 10 mg/kg in food and 5 mg/l (mg/kg) in beverages, a theoretical maximum dietary exposure of 0.75 mg/kg bw per day (45 mg/day for a 60 kg individual) was calculated.

Using the budget method developed by Denmark (Douglass et al., 1997) and an ADI of 0–5 mg/kg bw, as proposed by the sponsor, the Committee calculated the highest safe theoretical Benzoe Tonkinenesis concentrations in food and beverages. Assuming that solid food (3 kg) and beverages (6 litres) are each allocated half of the upper limit of the ADI, a maximum concentration for Benzoe Tonkinensis of 50 mg/kg in food and 25 mg/kg in beverages can be calculated. As each of these concentrations is higher than the proposed respective use levels, no further consideration of dietary exposure is necessary for the safety assessment of the

proposed uses. In light of the noted uses at levels above the proposed flavouring use levels, the Committee concluded that assessment of dietary exposure to Benzoe Tonkinensis at higher tiers would be needed.

3.3.3 Disappearance volume

The sponsor reported that Benzoe Tonkinensis is manufactured in the Lao People's Democratic Republic and exported for food use throughout the world. It reported disappearance volumes for the years 2000–2008. Over this period, 70 tonnes of Benzoe Tonkinensis were produced for export. Conservatively assuming that all of that material is sold in Europe for food use and applying the maximized survey-derived intake method used in the Procedure for the Safety Evaluation of Flavouring Agents, the Committee estimated a dietary exposure of 6 mg/person per day (0.1 mg/kg bw per day for a 60 kg individual).

3.3.4 Individual dietary records

The Committee reviewed an assessment of dietary exposure to Benzoe Tonkinensis that was prepared using food consumption data from the French 7-day second National Individual Survey on Food Consumption (INCA-2) (AFSSA, 2009). This survey gathered food and supplement intakes of 2624 adults (18–79 years of age) and 1455 children (3–17 years of age). The adult group was subdivided into three age groups: 18–34, 35–54 and 55–79 years; the children were also subdivided into three age groups: 3–10, 11–14 and 15–17 years. The survey was completed between 2005 and 2007. The sponsor considered the age grouping that gave the highest estimate of exposure for each food grouping. The 35- to 54-year-old grouping was used for all of the categories with the exceptions of the 55- to 79-year-old group for alcoholic beverages and the 18- to 34-year-old group for baked goods. The 3- to 10-year-old group was used for all food categories to estimate dietary exposure for children.

For adults, the average exposures ranged from 0.04 mg/day (frozen dairy) to 1.04 mg/day (alcoholic beverages), with a total of 3.16 mg/person per day (0.05 mg/kg bw per day for a 60 kg individual). The total for children was 2 mg/person per day (0.08 mg/kg bw per day for a 25 kg child). The 95th and 97.5th percentile exposures for adults were estimated to be 7.9 and 9.48 mg/person per day (0.1 and 0.2 mg/kg bw per day, respectively), respectively. For children, the 95th and 97.5th percentile exposures were 5 and 6 mg/person per day (both 0.2 mg/kg bw per day), respectively.

The Committee noted that the sponsor highlighted the use of "benzoin" without specific reference to Benzoe Tonkinensis in a number of countries, including Canada, Japan, Thailand and the USA. Use levels for some of these foods exceed the 10 and 5 mg/kg levels requested for food and beverage applications, respectively. High levels were noted for chewing gum applications, but the low chronic consumption of these foods results in estimates of Benzoe Tonkinensis exposure that are not significantly different from those reported herein for the flavour uses. Other foods, notably baked goods, can contain benzoin at levels up to 150 mg/kg. If Benzoe Tonkinensis is assumed to be the species in use, exposures of up to 30 mg/day might be seen, using the highly conservative assumption of

200 g of flavoured baked goods consumed daily. The Committee concluded that these potential exposures were not relevant to the current evaluation of Benzoe Tonkinensis as a general flavouring agent.

3.4 Conclusion

Chronic exposure to Benzoe Tonkinensis is expected to be below 0.2 mg/kg bw per day from the proposed uses. Putative uses of benzoin gum are not expected to significantly affect this estimate, nor would the use of Benzoe Tonkinensis in pharmaceutical or cosmetic applications.

4. COMMENTS

4.1 Toxicological data

The Committee considered the previous evaluations of three of the components of Benzoe Tonkinensis—namely, benzoic acid, vanillin and benzyl benzoate. At its forty-sixth meeting, the Committee confirmed the group ADI of benzyl derivatives, including benzoic acid and benzyl benzoate, of 0–5 mg/kg bw as benzoic acid equivalents (Annex 1, reference *122*), which was maintained at its fifty-seventh meeting (Annex 1, reference *154*). At its eleventh meeting, the Committee assigned vanillin an ADI of 0–10 mg/kg bw (Annex 1, reference *14*), which was maintained at the fifty-seventh meeting.

At the current meeting, the Committee evaluated toxicological studies with Benzoe Tonkinensis, including a 90-day oral toxicity study and genotoxicity assays.

In a 90-day oral toxicity study, rats were administered Benzoe Tonkinensis in corn oil by gavage at dose levels of 0, 500, 1000 or 2000 mg/kg bw per day; the study included two satellite groups of vehicle control and a high-dose group (2000 mg/kg bw per day) kept for an additional 28 days without any treatment. The Committee noted that the test material was inadequately characterized. There were no treatment-related effects on body weight, feed consumption, functional observational battery parameters, ophthalmology, haematology or gross pathology. Statistically significant increases in relative weights of the liver in female rats and of the liver, brain, heart and kidneys in male rats were observed in the high-dose group. Clinical biochemistry parameters showed dose-dependent alterations in ALT activity and total cholesterol level, reaching statistical significance at the highest dose. After the treatment was stopped, ALT activity and total cholesterol level returned to normal values at day 119, indicating reversibility of these effects. Histopathological findings of bile duct hyperplasia and multinucleated hepatocytes in the liver as well as lacteal ectasia (prominent white villi) in the jejunum were treatment related in both sexes in the intermediate-dose, high-dose and satellite high-dose groups. These findings correlated with the observed organ weight changes in liver. The Committee concluded that these effects in liver would not progress to neoplastic alterations. The NOAEL was considered to be 500 mg/kg bw per day.

The Committee concluded that Benzoe Tonkinensis was not mutagenic or clastogenic based on the results of the genotoxicity assays (reverse mutation tests in

bacteria, chromosomal aberration assay and a gene mutation assay in mammalian cells). However, the Committee noted that there was cytotoxicity in bacteria, mouse lymphoma cells and human lymphocytes at relatively low concentrations. The Committee also noted from published literature that some of the triterpenoids that were isolated from Benzoe Tonkinensis resin were cytotoxic to human leukaemic HL-60 cells. Benzoe Tonkinensis has not been tested in a carcinogenicity study.

4.2 Assessment of dietary exposure

The Committee received one submission for Benzoe Tonkinensis that contained information concerning food uses and estimated dietary exposure. Benzoe Tonkinensis can be used as a flavouring agent in solid foods and beverages. The sponsor stated that it has been used in foods in Europe. Benzoe Tonkinensis has a number of technical effects in food beyond use as a flavouring agent.

Benzoe Tonkinensis also finds use in pharmaceuticals and cosmetics. These uses were not considered by the Committee in the dietary exposure assessment, as cosmetic uses are not relevant to dietary exposure and pharmaceutical uses would not likely contribute significantly to overall chronic dietary exposure.

The Committee was requested to evaluate the use of Benzoe Tonkinensis as a flavouring agent in solid foods at levels up to 10 mg/kg and in beverages at levels up to 5 mg/kg. Using the budget method, assuming these use levels in all foods and beverages, the sponsor calculated a dietary exposure of 0.75 mg/kg bw per day for a 60 kg individual.

Benzoe Tonkinensis is manufactured predominantly in the Lao People's Democratic Republic and exported for food use throughout the world. The sponsor reported a total disappearance volume for the Lao People's Democratic Republic for the years 2000–2008 of 70 tonnes. Conservatively assuming that all of that material is sold in Europe for food use and applying the maximized survey-derived intake method, the Committee estimated a dietary exposure for Europe of 0.1 mg/kg bw per day for a 60 kg individual.

The Committee reviewed an assessment of dietary exposure to Benzoe Tonkinensis that was prepared using individual food consumption data from France and use levels submitted by the sponsor. This estimate included all uses in foods. For 60 kg adults, the mean exposure was 0.05 mg/kg bw per day, with a 95th percentile estimate of 0.1 mg/kg bw per day. The mean dietary exposure for children was 0.1 mg/kg bw per day for a 25 kg child, with a 95th percentile exposure of 0.2 mg/kg bw per day.

The Committee concluded that chronic dietary exposure to Benzoe Tonkinensis is expected to be below 0.2 mg/kg bw per day for children, the highest estimate including all uses in foods. As the levels of the individual components of Benzoe Tonkinensis are variable, the Committee did not enumerate exposure to each individual component, but the dietary exposure to benzoic acid, vanillin and benzyl benzoate would all be below 0.2 mg/kg bw per day, even if each comprised 100% of Benzoe Tonkinensis.

5. EVALUATION

The Committee noted that exposure to benzoic acid and benzyl benzoate from the use of Benzoe Tonkinensis is well below the upper limit of the group ADI (0–5 mg/kg bw) for benzyl derivatives, and exposure to vanillin is also well below the upper limit of its ADI (0–10 mg/kg bw). The Committee further noted that benzoic acid, one of the major components of Benzoe Tonkinensis, is used as a preservative, but that Benzoe Tonkinensis has not been assessed for this application. Comparing the conservative dietary exposure estimate for Benzoe Tonkinensis of 0.2 mg/kg bw per day with the NOAEL of 500 mg/kg bw per day identified in a 90-day oral toxicity study in rats, the margin of exposure is at least 2500. Because of the variability in composition of Benzoe Tonkinensis and the inadequate characterization of the material tested, the Committee concluded that the available data were inadequate to establish an ADI.

Considering the margin of exposure of 2500 for Benzoe Tonkinensis when used in food, the nature of the hepatic effects observed at doses above the NOAEL and the negative genotoxicity results, the Committee concluded that Benzoe Tonkinensis would not pose a health concern at current estimated dietary exposures, provided that it complies with the tentative specifications prepared at the current meeting, when used as a flavouring agent and in accordance with good manufacturing practice.

6. REFERENCES

AFSSA (2009). *Étude Individuelle Nationale des Consommations Alimentaires 2 (INCA 2) 2006–2007. Rapport. Septembre 2009 (version 2).* Maisons-Alfort, France, Agence française de sécurité sanitaire des aliments (http://www.anses.fr/Documents/PASER-Ra-INCA2.pdf).

Agroforex (2011). Data submitted to WHO by Agroforex Company, Vientiane, Lao People's Democratic Republic.

BIBRA (1989). *Toxicity profile. Gum benzoin*. The British Industrial Biological Research Association (BIBRA) Working Group, 5 pp.

Castel C et al. (2006a). Characterization of the chemical composition of a byproduct from Siam benzoin gum. *Journal of Agricultural and Food Chemistry*, 54(23):8848–8854.

Castel C et al. (2006b). Volatile constituents of benzoin gums: Siam and Sumatra (Part 2). Study of headspace sampling methods. *Flavour and Fragrance Journal*, 21:59–67.

Coskey RJ (1978). Contact dermatitis owing to tincture of benzoin. *Archives of Dermatology*, 114:128.

Douglass JS et al. (1997). Evaluation of the budget method for screening food additive intakes. *Food Additives and Contaminants*, 14:791–802.

Escarti A (2010a). *Bacterial reverse mutation test with Benzoe Tonkinensis*. Unpublished report No. B-00951 from Vivotecnia Research S.L., Tres Cantos (Madrid), Spain. Submitted to WHO by Agroforex Company, Vientiane, Lao People's Democratic Republic.

Escarti A (2010b). *Bacterial reverse mutation test with Benzoe Tonkinensis*. Unpublished report No. B-00883 from Vivotecnia Research S.L., Tres Cantos (Madrid), Spain. Submitted to WHO by Agroforex Company, Vientiane, Lao People's Democratic Republic.

Fernandez X et al. (2003). Volatile constituents of benzoin gums: Siam and Sumatra (Part 1). *Flavour and Fragrance Journal*, 18:328–333.

Fisher A (1982). Dermatitis of the hands from food additives. *Cutis*, 30:304–308, 312–318, 414.

Fox T (1874). Practical note on cutaneous subject. *Lancet*, 1:195.

Giridharrao PS et al. (2010). *Repeated dose 90-day oral toxicity study with Benzoe Tonkinensis in Wistar rats.* Unpublished report No. 09197 from International Institute of Biotechnology and Toxicology, Padappai, Tamil Nadu, India. Submitted to WHO by Agroforex Company, Vientiane, Lao People's Democratic Republic.

Margolin S (1970a). *Report to the Research Institute for Fragrance Materials.* 1 July [cited in Opdyke, 1973].

Margolin S (1970b). *Report to the Research Institute for Fragrance Materials.* 12 October [cited in Opdyke, 1973].

Mohan A (2010a). *In vitro cytogenetic assay measuring chromosomal aberration frequencies induced by Benzoe Tonkinensis in human lymphocytes.* Unpublished report No. 09198 from International Institute of Biotechnology and Toxicology, Padappai, Tamil Nadu, India. Submitted to WHO by Agroforex Company, Vientiane, Lao People's Democratic Republic.

Mohan A (2010b). *In vitro mammalian cell gene mutation assay of Benzoe Tonkinensis in L5178Y TK+/– mouse lymphoma cell line.* Unpublished report No. 09199 from International Institute of Biotechnology and Toxicology, Padappai, Tamil Nadu, India. Submitted to WHO by Agroforex Company, Vientiane, Lao People's Democratic Republic.

Opdyke DL (1973). Monographs on fragrance raw materials. *Food and Cosmetics Toxicology*, 11:871–872.

Scardamaglia L, Nixon R, Fewings J (2003). Compound tincture of benzoin: a common contact allergen? *Australasian Journal of Dermatology*, 44:180–184.

Sharp DW (1978). The sensitization potential of some perfume ingredients tested using a modified Draize procedure. *Toxicology*, 9:261–271.

Spott D, Shelley WB (1970). Exanthem due to contact allergen (benzoin) absorbed through skin. *Journal of the American Medical Association*, 214:1881–1882.

Wang F et al. (2006). Triterpenoids from the resin of *Styrax tonkinensis* and their antiproliferative and differentiation effects in human leukemia HL-60 cells. *Natural Products*, 69:807–810.

PONCEAU 4R (addendum)

First draft prepared by

U. Mueller,[1] M. DiNovi,[2] J.-C. Leblanc[3] and E. Vavasour[4]

[1] Food Standards Australia New Zealand, Canberra, Australia
[2] Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, Maryland, United States of America (USA)
[3] L'Agence nationale de la sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), Maisons-Alfort, France
[4] Ottawa, Ontario, Canada

1. EXPLANATION

Ponceau 4R (Chemical Abstracts Service No. 2611-82-7), also known as Cochineal Red and New Coccine, is a synthetic food colour. Ponceau 4R consists essentially of trisodium 2-hydroxy-1-(4-sulfonato-1-naphthylazo)-6,8-naphthalenedisulfonate and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Ponceau 4R was evaluated by the Committee at its present meeting at the request of the Codex Committee on Food Additives at its Forty-second Session (FAO/WHO, 2010). Ponceau 4R was previously evaluated by the Committee at its eighth, thirteenth, eighteenth, twenty-second, twenty-fifth and twenty-seventh meetings (Annex 1, references *8*, *19*, *35*, *47*, *56* and *62*). At its eighth meeting, the Committee did not establish an acceptable daily intake (ADI) for Ponceau 4R because of inadequate toxicological data but recognized that some long-term feeding studies were available. At its thirteenth meeting, the Committee reviewed these data and established a temporary ADI of 0–0.75 mg/kg body weight (bw) based on a no-observed-effect level (NOEL)[1] of 150 mg/kg bw per day in a long-term feeding study in rats. The ADI was made temporary because the Committee noted the absence of suitable information on the metabolism and kinetics of Ponceau 4R and a long-term feeding study in a second mammalian species. At its eighteenth meeting, the Committee considered an additional long-term feeding study in mice that had become available and revised the temporary ADI to 0–0.125 mg/kg bw based on a NOEL of 25 mg/kg bw per day (this NOEL assumed 500 mg/kg in the diet to be equivalent to 25 mg/kg bw per day). The current method of calculating administered dose from a concentration of test material present in the feed would yield an equivalent dose of 75 mg/kg bw per day (FAO/WHO, 2009). At that meeting, the Committee reiterated the need to review more studies on metabolism and reproduction and a long-term feeding study in a non-rodent species.

At the twenty-second and twenty-fifth meetings, the Committee extended the temporary ADI on the understanding that the data requested at the eighteenth meeting would become available for review. At the twenty-seventh meeting, the Committee reviewed new data on metabolism, a long-term study in rats that had been exposed in utero and through lactation, a multigeneration feeding study and a teratogenicity study. The Committee noted that the long-term study in rats showed no adverse effects in the kidneys and had a NOEL of 500 mg/kg bw per day based on reduced body weight gain at higher doses. The results of this study in rats and a reconsideration of the severity of the renal effects observed in the long-term study in mice led the Committee to establish an ADI of 0–4 mg/kg bw. The ADI was derived by applying a 100-fold safety factor to the higher NOEL from the mouse dietary study, which was equivalent to 375 mg/kg bw per day.

At its present meeting, the Committee based its evaluation on data previously reviewed together with a limited number of published studies that had become available since the twenty-seventh meeting. The new data included a

[1] At its sixty-eighth meeting (Annex 1, reference *187*), the Committee decided to differentiate between no-observed-effect level (NOEL) and no-observed-adverse-effect level (NOAEL). This NOEL would now be considered a NOAEL.

reproduction study in mice that measured several neurological end-points, studies on genotoxicity and biochemical enzyme activity, and studies on additive intolerance. The Committee took note of the content of a recently completed review of Ponceau 4R by the European Food Safety Authority (EFSA).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

No new information was available on the absorption, distribution and excretion of Ponceau 4R.

2.1.2 Biotransformation

Singh, Das & Khanna (1997) compared the rate of formation of aromatic amines from four red azo dyes—namely, Amaranth, Carmoisine, Fast Red E and Ponceau 4R (all at 37.5 µmol/l)—by gastrointestinal microbes under anaerobic conditions with that obtained using hepatic microsomes. The caecal suspension exhibited higher azo reductase activity compared with the hepatic microsomal fraction using each of the four azo dyes. Caecal microbes showed maximal azo reductase activity when Ponceau 4R was used as a substrate, followed by Fast Red E and Carmoisine, whereas the activity was least with Amaranth. Similarly, maximum hepatic microsomal azo reductase activity was observed with Ponceau 4R as the substrate, followed by Fast Red E and Carmoisine, whereas the least activity was observed with Amaranth. Caecal flora possessed almost a 17-fold higher capability to degrade Ponceau 4R and Fast Red E colorants compared with the hepatic microsomal fraction.

2.1.3 Effects on enzymes and other biochemical parameters

To investigate the inhibition of the activities of human phenolsulfotransferase-P (PST-P), phenolsulfotransferase-M (PST-M) and monoamine oxidase A and B by eight food colours, including Ponceau 4R and Sunset Yellow FCF, each colour was tested separately at a concentration of 1, 5 or 25 µmol/l using conventional in vitro testing protocols. The substrates used for the enzymes were phenol for PST-P, tyramine for PST-M and [^{14}C]tyramine for both monoamine oxidases. At a concentration of 25 µmol/l, Ponceau 4R and Sunset Yellow FCF completely inhibited PST-P activity. However, at 5 and 1 µmol/l, the extent of inhibition was 39% and 11%, respectively, for Ponceau 4R and 55% and 17%, respectively, for Sunset Yellow FCF. Ponceau 4R and Sunset Yellow FCF had little to no inhibitory effect on PST-M or monoamine oxidase activities at a concentration of 25 µmol/l (Gibb, Glover & Sandler, 1987).

Kuno & Mizutani (2005) investigated the influence of Ponceau 4R (New Coccine) on the activities of phase I and phase II drug-metabolizing enzymes (cytochrome P450 [CYP] 2A6, uridine diphosphate glucuronosyltransferase [UGT] 1A6 and 2B7) derived from bovine liver microsomes. Their findings indicated that Ponceau 4R is neither a substrate nor an inhibitor of the enzymes studied.

2.2 Toxicological studies

2.2.1 Acute toxicity

No new information was available on the acute toxicity of Ponceau 4R.

2.2.2 Short-term studies of toxicity

No new information was available from short-term studies of the toxicity of Ponceau 4R.

2.2.3 Long-term studies of toxicity and carcinogenicity

No new information was available from long-term studies of the toxicity and carcinogenicity of Ponceau 4R.

2.2.4 Genotoxicity

The genotoxicity of Ponceau 4R is summarized in Table 1.

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration study

Ponceau 4R admixed in the diet was fed to Crj: CD-1 mice at concentrations of 0 (control), 1200, 2400 or 4800 mg/kg (equal to 0, 212, 423 and 819 mg/kg bw per day; average for both sexes combined) from 5 weeks of age in the F_0 generation to 9 weeks of age in the F_1 generation. Feed consumption data indicated no significant difference between controls and those groups consuming Ponceau 4R. Mice were weighed on days 0, 2, 4, 7, 14, 21, 28 and 30 during the pre-mating phase. Females were paired 1:1 with males and separated after 5 days. Dams were allowed to deliver and rear their offspring in solitude. Pups were weighed on postnatal days (PNDs) 0, 4, 7, 14 and 21. Functional and behavioural parameters, such as surface righting (PNDs 4 and 7), negative geotaxis (body righting on an inclined plane; PNDs 4 and 7), cliff avoidance (PND 7), swimming behaviour (PNDs 4 and 14) and olfactory orientation (PND 14), were measured in all F_1 pups during PNDs 0–21. On PND 49, all pups performed in a multiple water T-maze daily for 3 consecutive days.

There was no adverse effect of Ponceau 4R on litter size, litter weight or sex ratio at birth. The average body weight of male and female offspring was increased significantly in the high-dose group at PNDs 0, 4 and 21. In behavioural developmental parameters, surface righting at PND 4, but not at PND 7, was affected significantly in the high-dose group in male offspring. Other variables measured showed no consistently significant adverse effect on either sex in the lactation period. In multiple water T-maze performances in the F_1 generation, the time taken was significantly longer in the middle-dose and high-dose groups in males compared with the controls, and those effects were significantly dose related ($P < 0.01$). It was concluded that the no-observed-adverse-effect level (NOAEL) was 1200 mg/kg in the diet (approximately 205 mg/kg bw per day) for maze learning by males in the F_1 generation (Tanaka, 2006).

Table 1. Genotoxicity of Ponceau 4R

End-point	Test system	Concentration	Result	Reference
In vitro				
Forward mutation	Mouse lymphoma L5178Y cells, $tk^{+/-}$ locus	Up to 10 000 µg/ml, ±S9	Negative	Cameron et al. (1987)
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	Up to 10 000 µg/ml (liquid culture method), ±S9	Negative	Cameron et al. (1987)
	S. typhimurium TA98, TA100, TA1535, TA1537 and TA1538	Up to 5000 µg/ml (liquid culture method), ±S9	Negative	Longstaff et al. (1984)
	S. typhimurium TA92, TA94, TA98, TA100, TA1535 and TA1537	Up to 5000 µg/plate, ±S9	Negative	Ishidate et al. (1984)
	S. typhimurium TA1535 and TA1538 and *Escherichia coli* WP2uvrA	Up to 10 000 µg/ml (liquid culture method), ±S9	Negative	Haveland-Smith & Combes (1980)
Chromosomal aberration	Chinese hamster fibroblast line	Up to 1000 µg/ml, –S9, 24 h and 48 h incubation	Positive	Ishidate et al. (1984)
DNA repair	Rat hepatocytes	Up to 0.1 mmol/l, 4 h incubation	Negative	Kornbrust & Barfknecht (1985)
In vivo				
DNA repair	Hepatocytes taken from oral gavage–dosed Sprague-Dawley rat	300 mg/kg bw	Negative	Kornbrust & Barfknecht (1985)
Comet assay	ddY mouse (oral gavage); glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow examined	1–2000 mg/kg bw with 3 h exposure or 2000 mg/kg bw with 24 h exposure	Positive: colon, >10 mg/kg bw; stomach, bladder, >100 mg/kg bw; kidney, liver, lung, bladder, 2000 mg/kg bw for 24 h	Tsuda et al. (2001); Sasaki et al. (2002)
	ICR mouse or F344 rat (oral gavage dosed); glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow examined	Mouse; 1 or 10 mg/kg bw with 3 h exposure Rat; 10 mg/kg bw with 3 h exposure	Positive: mouse, colon at 10 mg/kg bw Negative: rat	Shimada et al. (2010)

DNA, deoxyribonucleic acid; S9, 9000 × *g* supernatant from rat liver

(b) Developmental toxicity

No new information was available on the developmental toxicity of Ponceau 4R.

2.3 Observations in humans

2.3.1 Case–control studies

Common clinical signs attributed to food intolerance often involve recurrent urticaria or angio-oedema, functional upper and/or lower gastrointestinal disturbances or nonspecific symptoms such as headache, nausea and lassitude. However, many of the reports on food colour intolerance are characterized by poorly controlled challenge procedures (Mikkelsen et al., 1978; Ibero et al., 1982). Studies performed under properly controlled conditions imply that intolerance to food additives in patients with chronic urticaria or angio-oedema is uncommon (Supramaniam & Warner, 1986; Simon, 2003). The true prevalence estimates range around 0.03–2% (Weber et al., 1979; Hannuksela & Haahtela, 1987; Young et al., 1987; Fuglsang et al., 1994).

Veien & Krogdahl (1991) reported a case of a 24-year-old woman who responded with development of leukoclastic vasculitis (i.e. inflammation of small blood vessels) after a placebo-controlled oral challenge with 50 mg of Ponceau 4R.

2.3.2 Clinical trials

Bateman et al. (2004) investigated the behavioural effects on 3-year-old children (n = 277) of ingesting a high-dose azo food dye mixture containing Sunset Yellow FCF, Tartrazine, Carmoisine and Ponceau 4R (5 mg of each) and 45 mg sodium benzoate in a double-blind, placebo-controlled study. The children were classified as having hyperactivity (HA) (using two different activity scales: emotionality, activity and sociability; and Weiss–Werry–Peters) or not, with or without atopy (AT) (i.e. positive skin prick test with a number of known protein allergens), in a 2 × 2 group design (AT/HA, non-AT/HA, AT/non-HA, non-AT/non-HA). Over a 4-week period, the children received either the azo dye mixture with fruit juice or placebo (fruit juice only) on the 2nd and 4th weeks. The children's behaviour was assessed by research psychologists using validated tests and by the parents. Using assessments made by the parents, there were significant reductions in hyperactive behaviour during the withdrawal phase. Furthermore, there were significantly greater increases in hyperactive behaviour during the active period compared with the placebo period. These effects were not influenced by the presence or absence of previously diagnosed hyperactivity or by the presence or absence of atopy. However, there were no significant differences detected based on objective interactive testing by psychologists in the clinic.

A follow-up study was conducted to further investigate the association of ingestion of a mixture of food colour additives and sodium benzoate with hyperactive behaviour in children. The hypothesis was tested using a community-based, double-blind, placebo-controlled randomized crossover food challenge in which two groups of children aged 3 (n = 153) and 8 or 9 years (n = 144) received one of two mixtures

of four food colour additives and sodium benzoate in a fruit drink administered at home by a parent. The children were self-identified from the general population and represented a range of behaviour from normal to hyperactive. All of the food colour additives except Quinoline Yellow were azo dyes. The food additives comprising mixture A (Sunset Yellow, Carmoisine, Tartrazine and Ponceau 4R in unequal proportions plus sodium benzoate) were those tested in the Bateman et al. (2004) study, whereas mixture B (Sunset Yellow, Carmoisine, Quinoline Yellow and Allura Red in equal proportions plus sodium benzoate) reflected a mixture considered representative for sweets as they are consumed by children in the United Kingdom. On a body weight basis, the total dose of colour additives received by the 3-year-old children was 1.33 mg/kg bw per day from mixture A and 2.0 mg/kg bw per day from mixture B. For the 8- or 9-year-old children, the total dose was 0.8 mg/kg bw per day from mixture A and 2.0 mg/kg bw per day from mixture B. For sodium benzoate, the younger age group received a dose of 3 mg/kg bw per day from each mixture, whereas the older children received only 1.45 mg/kg bw per day. Behaviour was assessed through a novel global hyperactivity aggregate (GHA) measure, which comprised an unweighted aggregate of standardized scores from validated attention deficit hyperactivity disorder (ADHD) behaviour assessment tools. Behaviour at home was assessed by parents and in school by teachers and independent observers for both age groups. An additional computer-based tool was used to assess behaviour for the 8- to 9-year-old group. A high GHA score indicated greater hyperactivity.

Ingestion of the fruit drink with mixture A, but not mixture B, significantly increased GHA scores for all 3-year-old children relative to the placebo control GHA scores and for the high-consumption subsets (high-consumption subsets consist of children who had consumed ≥85% of the drinks in each treatment week). For the 8- and 9-year-olds, a significant increase in GHA scores was not observed in either the entire sample or the high-consumption subset with mixture A relative to placebo, whereas significant increases in the entire group and the high-consumption subset were observed for mixture B. The magnitudes of the changes in GHA scores associated with the active challenges were small, with the effect sizes averaging about 0.18. This is approximately equivalent to less than a 10% difference between children with ADHD and children without that disorder. Variability in the results may have been introduced by the nearly 2-fold difference in doses of colour additives received by the 3-year-old children compared with the 8- and 9-year-old children and the 2-fold difference in the dose of colour additives received by the 8- and 9-year-old children consuming mixture A compared with mixture B. In addition, inconsistency in the timing of treatment relative to the observation of behaviour could have introduced variability in the context of the comment by the study authors that onset of hyperactive behaviour in response to food additives can be produced within 1 hour of consumption (McCann et al., 2007).

In order to investigate the hypothesis that the children's behaviour reported in the McCann et al. (2007) study was influenced by allelic variation in a number of genes that have previously been implicated in ADHD (Thapar et al., 1999; Swanson et al., 2000; Kuntsi & Stevenson, 2001), buccal swabs were collected for genotypic analyses of cellular deoxyribonucleic acid (DNA). The genes studied were from the dopamine (dopamine transporter [DAT1], dopamine D4 receptor [DRD4]

and catechol *O*-methyl-transferase [COMT]), adrenergic (adrenergic receptor alpha 2A [ADRA2A]) and histamine (histamine *N*-methyl-transferase [HNMT]) neurotransmitter systems. The genotype analysis involved the detection of single nucleotide polymorphisms (two in HNMT, one in COMT, one in DRD4 and one in ADRA2A) in the genes. There was evidence that the HNMT T939C and the DRD4 4rs740373 polymorphisms correlated to the overall GHA score in the 3-year-old children. However, there was no significant relationship of the polymorphisms to the GHA scores in the 8- and 9-year-olds (Stevenson et al., 2010).

3. DIETARY EXPOSURE

3.1 Introduction

The Committee has not previously evaluated dietary exposure estimates for Ponceau 4R. The Committee received a submission from EFSA concerning dietary exposure to Ponceau 4R that was a part of its re-evaluation of the safety of a number of artificial colours (EFSA, 2009). Additionally, the Committee accessed and considered the dietary exposure sections of a 2008 report from Food Standards Australia New Zealand (FSANZ) on artificial colours (FSANZ, 2008).

3.1.1 Food uses

Ponceau 4R is used to colour both solid foods and beverages. In the European Union (EU), its use is permitted at the maximum levels shown in Table 2. Under the Australia New Zealand Food Code, Ponceau 4R is permitted at levels up to 70 mg/kg in beverages and 290 mg/kg in other foods.

3.2 International estimates of dietary exposure

The Committee concluded that international estimates of dietary exposure to Ponceau 4R made using Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diet information would not be appropriate, as Ponceau 4R is always used at low levels in highly processed foods.

3.3 National estimates of dietary exposure

3.3.1 European Food Safety Authority

The 2009 EFSA report on the re-evaluation of Ponceau 4R (E 124) as a food additive contained a thorough examination of dietary exposure to this colour. The analysis used a tiered approach, beginning with a budget screening method and continuing with additional refined estimates.

(a) Budget method

EFSA used a budget method (tier 1 approach) as described in the report of the Scientific Cooperation (SCOOP) Task 4.2 (EC, 1998). The generalized equation for the budget method is shown below.

Table 2. Maximum permitted use levels of Ponceau 4R in beverages and foodstuffs in the EU

Beverages	Maximum permitted level (mg/l)
Non-alcoholic flavoured drinks	50
Americano Bitter soda, bitter vino Liquid food supplements/dietary integrators	100
Spirituous beverages Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails Fruit wines, cider and perry	200
Foodstuffs	**Maximum permitted level (mg/kg)**
Confectionery Fine bakery wares Edible ices Desserts including flavoured milk products Complete formulae for weight control intended to replace total daily food intake or an individual meal Complete formulae and nutritional supplements for use under medical supervision Soups	50
Flavoured processed cheese Fish paste and crustacean paste Smoked fish Savoury snack products and savoury coated nuts Meat and fish analogues based on vegetable proteins Jam, jellies and marmalades and other similar fruit preparations including low-calorie products	100
Candied fruit and vegetables, mostarda di frutta Preserves of red fruits Extruded or expanded savoury snack products Sobrasada Chorizo sausage	200
Pre-cooked crustaceans Salchichon	250
Mustard Fish roe Solid food supplements/dietary integrators	300
Decorations and coatings Sauces, seasonings, pickles, relishes, chutney and piccalilli Salmon substitutes Surimi	500
Edible cheese rind and edible casings	Quantum satis

EFSA assumed that the maximum permitted use levels considered were 200 mg/l for beverages and 500 mg/kg for solid foods. The default proportion of beverages and solid food that could contain the additive (25%) was considered adequate. Thus, a typical adult weighing 60 kg might consume 1.5 litres of coloured beverages and 375 g of coloured solid foods containing Ponceau 4R, daily. The theoretical maximum daily exposure for adults would be:

(200 mg/l beverage × 0.1 litre beverage/kg bw × 0.25) + (500 mg/kg food × 0.025 kg food/kg bw × 0.25) = 5 + 3.125 = 8.1 mg/kg bw per day

A similar calculation was carried out for children, assuming that the maximum level in beverages was 50 mg/l (after exclusion of alcoholic drinks). It was further assumed that 100% of beverages consumed could be coloured. The theoretical maximum daily exposure for children would be:

(50 mg/l beverage × 0.1 litre beverage/kg bw × 1) + (500 mg/kg food × 0.025 kg food/kg bw × 0.25) = 5 + 3.125 = 8.1 mg/kg bw per day

(b) Refined estimates

Exposure estimates for children 1–10 years of age were performed based on detailed individual food consumption data from eight European countries (Belgium, Czech Republic, Finland, France, Germany, Italy, the Netherlands and Spain). Estimates for children aged 1.5–4.5 years in the United Kingdom were made using detailed individual food consumption data from the United Kingdom National Diet and Nutrition Survey (1992–1993) and maximum permitted levels of use as specified in the EU Directive 94/36/EC on food colours (EU, 1994) (tier 2 approach). The United Kingdom population was considered as representative of all EU adults for the Ponceau 4R exposure estimates, as it was considered to be the population with the highest consumption of soft drinks in Europe. Additionally, the adult food consumption data for the maximum permitted levels population were considered to be more refined than those available from the EFSA Concise European Food Consumption Database.

The mean dietary exposure estimates for European children aged 1–10 years and weighing 25–30 kg when considering maximum permitted levels of use ranged from 0.3 to 2.5 mg/kg bw per day, whereas those at the 95th percentile ranged from 0.7 to 6.7 mg/kg bw per day. For United Kingdom children aged 1.5–4.5 years and weighing 15 kg, the mean dietary exposure was 1.4 mg/kg bw per day, and dietary exposure at the 97.5th percentile[1] was 3.5 mg/kg bw per day. Estimates reported for the United Kingdom adult population were 0.5 mg/kg bw per day at the mean and 1.1 mg/kg bw per day at the 97.5th percentile. For adults, the main contributors to the total anticipated exposure (>10%) were soft drinks (40%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli (14%) and fruit wines, cider and perry (13%).

[1] The United Kingdom 97.5th percentile estimates herein are made from the 97.5th percentile estimate from beverages combined with the per capita estimates from all other coloured foods.

The tier 3 approach employed by EFSA used maximum reported Ponceau 4R use levels in place of the maximum permitted levels of tier 2. In some, but not all, cases, these were lower than the levels used in tier 2. In this analysis, the dietary exposures to Ponceau 4R for European children ranged from 0.3 to 2.4 mg/kg bw per day at the mean and from 0.7 to 6.2 mg/kg bw per day at the 95th percentile. For United Kingdom children aged 1.5–4.5 years, the mean dietary exposure was 1.3 mg/kg bw per day, and dietary exposure at the 97.5th percentile was 3.3 mg/kg bw per day. Estimates for the United Kingdom adult population were 0.4 mg/kg bw per day at the mean and 1.0 mg/kg bw per day at the 97.5th percentile. As in the tier 2 estimates above, for adults, the main contributors to the total anticipated exposure (>10%) were soft drinks (52%) and sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli (16%).

The results of the EFSA tiered approach analyses are summarized in Table 3.

3.3.2 Food Standards Australia New Zealand

FSANZ included Ponceau 4R in an overall survey of artificial colour use in foods in 2006. The foods and beverages examined were confectionery, ice cream, cheese, yoghurt, margarine, flavoured milk, flavoured soya beverages, soft drinks, cordials, fruit drinks, alcoholic drinks, biscuits, cakes, pastries, savoury snacks, breakfast cereals, pre-prepared meals, processed meats, sauces, toppings, jams/conserves and jelly. A small number of products that claimed to contain “no added colours” or “no artificial colour” were also sampled.

Assessments of dietary exposure to Ponceau 4R were made for the Australian population aged 2 years and above, children aged 2–5 years, children aged 6–12 years, adolescents aged 13–18 years, adults aged 19–24 years and adults aged 25 years and above. The dietary exposures were estimated by combining usual patterns of food consumption, as derived from the 1995 National Nutrition Survey, with analysed levels of the colour in foods. Estimates were made using two scenarios: the mean colours scenario and the maximum colours scenario.

In the mean colours scenario, mean analytical concentrations of Ponceau 4R in survey foods were used. Both detected and “non-detect” results were used to derive the mean analytical concentrations. It was assumed that the use of mean food colour concentrations represents the most realistic exposure for consumers of a range of brands and varieties of particular foods over a period of time. In the maximum colours scenario, estimates were made by using the maximum analytical concentrations of Ponceau 4R in the survey foods. The use of maximum food colour concentrations assumed that every processed food consumed contained the highest concentration of each colour detected in the survey, in this case, Ponceau 4R. The report states that this model will significantly overestimate exposure to added colours, except where products containing food colours at the highest levels of use are consumed every day. The estimates made using the maximum colours scenario were not used by FSANZ in its overall evaluation of the safety of the use of artificial colours.

For the Australian population aged 2 years and older, the mean dietary exposure to Ponceau 4R was 0.15 mg/day, with a 90th percentile exposure of

Table 3. EFSA dietary exposures to Ponceau 4R

	Exposure (mg/kg bw per day)		
	Adults	Children 1.5–4.5 years old	Children 1–10 years old
Budget method	8.1		8.1[a]
Maximum permitted levels			
- Mean exposure	0.5	1.4	0.3–2.5
- Exposure at the 95th or 97.5th percentile	1.1	3.5	0.6–6.7
Maximum reported use levels			
- Mean exposure	0.4	1.3	0.3–2.4
- Exposure at the 95th or 97.5th percentile	1.0	3.3	0.7–6.2

[a] For children (age range not specified).

0.45 mg/day. The highest subpopulation mean was 0.22 mg/day for 13- to 18-year-olds. The highest subpopulation 90th percentile exposure was 0.62 mg/day, also for 13- to 18-year-olds. The highest estimates made using the maximum colours scenario were 1.17 mg/day at the mean and 2.90 mg/day at the 90th percentile, both for the 13- to 18-year-old subpopulation. The main contributors to dietary exposure were ice cream and ice confections, cakes, muffins and pastries, and soft drinks.

These results are summarized in Table 4.

3.4 Conclusions

The estimates of dietary exposure to Ponceau 4R calculated by EFSA were much higher than those of FSANZ. The Committee concluded that this was due to EFSA's use of maximum permitted and maximum reported use levels in its tier 2 and tier 3 approaches, as opposed to FSANZ's use of the mean analysed levels for all foods. The latter approach is considered to be more realistic for preparing lifetime dietary exposure estimates. The Committee concluded that 6 mg/kg bw per day, the tier 3, 97.5th percentile EFSA estimate for children 1–10 years of age, should be considered for use in the safety assessment for Ponceau 4R, as it represents the most conservative assessment. However, it recognized that the FSANZ estimate for children, 0.02 mg/kg bw per day, was a more realistic dietary exposure estimate because of the extensive post-market analyses used in its preparation.

4. COMMENTS

4.1 Toxicological data

This summary of the available toxicological data combines the studies previously reviewed (Annex 1, references *8*, *19*, *35*, *47*, *56* and *62*) with recently published data.

Table 4. FSANZ dietary exposures to Ponceau 4R using the mean colours scenario

Population group	Mean exposure		90th percentile exposure	
	mg/person per day	mg/kg bw per day	mg/person per day	mg/kg bw per day
2–5 years old	0.12	0.01	0.38	0.02
6–12 years old	0.21	0.01	0.56	0.02
13–18 years old	0.22	<0.01	0.62	0.01
19–24 years old	0.17	<0.01	0.49	0.01
25+ years old	0.13	<0.01	0.38	0.01
2+ years old	0.15	<0.01	0.45	0.01

The absorption of ingested Ponceau 4R is limited. After Ponceau 4R is anaerobically reduced by microflora in the gastrointestinal tract, small amounts of its metabolites, in the form of the free sulfonated aromatic amines, naphthionic acid and 7-hydroxy-8-amino-naphthalene-1,3-disulfonic acid, reach the systemic circulation. Ponceau 4R does not accumulate in tissues. Almost all of an orally administered dose is excreted in urine and faeces within 72 hours, with the majority (90%) being present in faeces.

Repeated-dose feeding studies of short and long duration revealed no adverse findings. In 90-day studies, NOAELs of 500 mg/kg bw per day in rats and 300 mg/kg bw per day in pigs were reported. For long-term daily exposure, the NOAELs were 375 mg/kg bw per day in mice and 500 mg/kg bw per day in rats.

There was no evidence of carcinogenicity in long-term feeding studies in rats at doses up to 1500 mg/kg bw per day and in mice at doses up to 1875 mg/kg bw per day. Despite a recent report of a comet assay showing evidence of DNA damage in the colon and bladder at 10 mg/kg bw and in the stomach at 100 mg/kg bw, there was no evidence of any neoplasia in the stomach, bladder or colon of mice in the carcinogenicity studies. The authors of the comet assay study noted that a histopathological examination did not reveal any treatment-related effects in the colon, bladder or glandular stomach. No mutagenic or cytotoxic effects were found when Ponceau 4R was tested in a range of in vitro experiments.

Reproduction studies revealed no adverse effects of Ponceau 4R in the feed at doses equivalent to 1250 mg/kg bw per day in the rat and up to 205 mg/kg bw per day in a neurobehavioural study in mice. Adverse neurobehavioural findings among mouse pups were inconsistent. Teratogenicity studies in mice at oral gavage doses up to 100 mg/kg bw per day (the highest dose tested) and in rats at 4000 mg/kg bw per day did not reveal any adverse effects.

Urticarial and vasculitic reactions have been reported in humans following exposure to Ponceau 4R. However, most of these reports are characterized by poorly controlled challenge procedures. Although recent studies performed with

better control conditions are available, no conclusion on idiosyncratic responses to Ponceau 4R could be drawn from the available evidence.

The administration of six different food colours and a preservative, sodium benzoate, and the presence of multiple methodological deficiencies limited the value of a recent study that investigated a possible relationship between hyperactivity in children and the consumption of beverages containing food colours. The use of mixtures in dosing studies does not permit any observed effects to be ascribed to individual components.

4.2 Assessment of dietary exposure

Estimates of dietary exposure to Ponceau 4R prepared and published by EFSA and FSANZ were available to the Committee. The estimates of dietary exposure to Ponceau 4R calculated by EFSA were much higher than those of FSANZ (0.02 mg/kg bw per day at the 90th percentile for children). The Committee concluded that this was due to the use of maximum reported use levels by EFSA, as opposed to the use of the mean analysed levels for all foods by FSANZ. The latter approach is considered to be more realistic for estimating lifetime dietary exposure. Because of the conservative assumptions used by EFSA in making the exposure estimates, the Committee concluded that the 97.5th percentile estimate of 6 mg/kg bw per day for children should be considered in the safety assessment for Ponceau 4R in addition to the more realistic FSANZ estimate.

5. EVALUATION

The Committee noted that the data do not indicate a need to revise the existing ADI of 0–4 mg/kg bw for Ponceau 4R. The Committee noted that EFSA's conservative 97.5th percentile dietary exposure for children was above the ADI, whereas the 90th percentile dietary exposure for children estimated by the more realistic FSANZ approach was 0.5% of the upper limit of the ADI. In consequence, the Committee concluded that the dietary exposure of children to Ponceau 4R does not present a health concern.

6. REFERENCES

Bateman B et al. (2004). The effects of a double-blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. *Archives of Disease in Childhood*, 89:506–511.

Cameron TP et al. (1987). Mutagenic activity of 27 dyes and related chemicals in the *Salmonella*/microsome and mouse lymphoma TK+/– assays. *Mutation Research*, 189:223–261.

EC (1998). *Report on methodologies for the monitoring of food additive intake across the European Union. Final report submitted by the Task Coordinator, 16 January 1998.* Reports of a Working Group on Scientific Cooperation on Questions Relating to Food, Task 4.2. Brussels, Belgium, European Commission, Directorate General III Industry (SCOOP/INT/REPORT/2).

EFSA (2009). Scientific opinion on the re-evaluation of Ponceau 4R (E 124) as a food additive. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). *The EFSA [European Food Safety Authority] Journal*, 7(11):1328 [39 pp.] (http://www.efsa.europa.eu/en/efsajournal/pub/1328.htm; accessed 29 May 2011).

EU (1994). European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. *Official Journal of the European Communities*, L 237:13–29.

FAO/WHO (2009). Annex 2: Dose conversion table. In: *Principles and methods for the risk assessment of chemicals in food*. Geneva, Switzerland, Food and Agriculture Organization of the United Nations and World Health Organization (Environmental Health Criteria 240; http://whqlibdoc.who.int/ehc/WHO_EHC_240_14_eng_Annex2.pdf).

FAO/WHO (2010). *Report of the Forty-second Session of the Codex Committee on Food Additives, Beijing, China, 15–19 March 2010.* Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (ALINORM 10/33/12; http://www.codexalimentarius.net/web/archives.jsp?year=10).

FSANZ (2008). *Survey of added colours in foods available in Australia. Study of concentrations in foods including dietary exposure assessment and risk characterisation.* Food Standards Australia New Zealand (http://www.foodstandards.gov.au/_srcfiles/Colours%20Survey_Final%20Report%2022%20Oct%2008%20_2_.pdf; accessed 29 May 2011).

Fuglsang G et al. (1994). Adverse reactions to food additives in children with atopic symptoms. *Allergy*, 49:31–37.

Gibb C, Glover V, Sandler M (1987). In vitro inhibition of phenolsulphotransferase by food and drink constituents. *Biochemical Pharmacology*, 36:2325–2330.

Hannuksela M, Haahtela T (1987). Hypersensitivity reactions to food additives. *Allergy*, 42:561–575.

Haveland-Smith RB, Combes RD (1980). Screening of food dyes for genotoxic activity. *Food and Cosmetics Toxicology*, 18:285–290.

Ibero M et al. (1982). Dyes, preservatives and salicylates in the induction of food intolerance and/or hypersensitivity in children. *Allergologia et Immunopathologia*, 10:263–268.

Ishidate M et al. (1984). Primary mutagenicity screening of food additives currently used in Japan. *Food and Chemical Toxicology*, 22:623–636.

Kornbrust D, Barfknecht T (1985). Testing of 24 food, drug, cosmetic, and fabric dyes in the in vivo/in vitro rat hepatocyte primary culture/DNA repair assays. *Environmental Mutagenesis*, 7:101–120.

Kuno N, Mizutani T (2005). Influence of synthetic and natural food dyes on activities of CYP2A6, UGT1A6, and UGT2B7. *Journal of Toxicology and Environmental Health, Part A*, 68:1431–1444.

Kuntsi J, Stevenson J (2001). Psychological mechanisms in hyperactivity: II. The role of genetic factors. *Journal of Child Psychology and Psychiatry*, 42:211–219.

Longstaff E et al. (1984). A comparison of the predictive values of the *Salmonella*/microsome mutation and BHK21 cell transformation assays in relation to dyestuffs and similar materials. *Dyes and Pigments*, 5:65–82.

McCann D et al. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomized, double-blinded, placebo-controlled trial. *Lancet*, 370:1560–1567.

Mikkelsen H et al. (1978). Hypersensitivity reactions to food colours with special reference to the natural annatto extract (butter colour). *Archives of Toxicology: Supplement*, 1:141–143.

Sasaki YF et al. (2002). The comet assay with 8 mouse organs: results with 39 currently used food additives. *Mutation Research*, 519:103–119.

Shimada C et al. (2010). Differential colon DNA damage induced by azo food additives between rats and mice. *Journal of Toxicological Sciences*, 35:547–554.

Simon RA (2003). Adverse reactions to food additives. *Current Allergy and Asthma Reports*, 3:62–66.

Singh S, Das M, Khanna SK (1997). Comparative azo reductase activity of red azo dyes through caecal and hepatic microsomal fraction in rats. *Indian Journal of Experimental Biology*, 35:1016–1018.

Stevenson J et al. (2010). The role of histamine degradation gene polymorphisms in moderating the effects of food additives on children's ADHD symptoms. *American Journal of Psychiatry*, 167:1108–1115.

Supramaniam G, Warner JO (1986). Artificial food additive intolerance in patients with angio-oedema and urticaria. *Lancet*, 2:907–909.

Swanson JM et al. (2000). Dopamine genes and ADHD. *Neuroscience and Biobehavioural Reviews*, 24:21–25.

Tanaka T (2006). Reproductive and neurobehavioural toxicity study of Ponceau 4R administered to mice in the diet. *Food and Chemical Toxicology*, 44:1651–1658.

Thapar A et al. (1999). Genetic basis of attention deficit and hyperactivity. *British Journal of Psychiatry*, 174:105–111.

Tsuda S et al. (2001). DNA damage induced by red food dyes orally administered to pregnant and male mice. *Toxicological Sciences*, 61:92–99.

Veien NK, Krogdahl A (1991). Cutaneous vasculitis induced by food additives. *Acta Dermato-Venereologica*, 71:73–74.

Weber RW et al. (1979). Incidence of bronchoconstriction due to aspirin, azo dyes, non-azo dyes, and preservatives in a population of perennial asthmatics. *Journal of Allergy and Clinical Immunology*, 64:32–37.

Young E et al. (1987). The prevalence of reaction to food additives in a survey population. *Journal of the Royal College of Physicians*, 21:241–247.

PULLULANASE FROM BACILLUS DERAMIFICANS *EXPRESSED IN* BACILLUS LICHENIFORMIS

First draft prepared by

U. Mueller,[1] M. DiNovi,[2] A. Mattia,[2] I. Meyland[3] and Z. Olempska-Beer[2]

[1] Food Standards Australia New Zealand, Canberra, Australia
[2] Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, Maryland, United States of America (USA)
[3] Technical University of Denmark, Søborg, Denmark

1. EXPLANATION

At the request of the Codex Committee on Food Additives at its Forty-second Session (FAO/WHO, 2010), the Committee evaluated the enzyme pullulanase (pullulan 6-α-glucanohydrolase; Enzyme Commission No. 3.2.1.41) derived from a genetically modified strain of *Bacillus licheniformis*. The Committee had previously evaluated pullulanase from *Klebsiella aerogenes* at its twenty-fifth meeting (Annex 1, reference *56*). Pullulanase catalyses the hydrolysis of the (1,6-α-D) glucosidic linkages in liquefied starch to produce linear oligosaccharides. It is used in the manufacture of starch hydrolysates (maltodextrins, maltose and glucose) and high-fructose corn syrup and in the production of beer and potable alcohol.

1.1 Genetic modification

Pullulanase is manufactured by pure culture fermentation of a genetically modified strain of *Bacillus licheniformis* containing the pullulanase gene from *Bacillus deramificans*. *Bacillus licheniformis* is a Gram-positive bacterium that is widely distributed in nature and is considered to be non-pathogenic and non-toxigenic. *Bacillus licheniformis* has a long history of use in the production of enzymes used in food processing, including enzymes from genetically engineered strains of the organism. *Bacillus licheniformis* has been granted a Qualified Presumption of Safety status by the European Food Safety Authority (EFSA).

Prior to the introduction of the pullulanase gene, the *B. licheniformis* host strain was genetically modified through deletion of its sporulation capability, amylase activity and chloramphenicol acetyltransferase activity. The modified host strain was then transformed with an amplifiable deoxyribonucleic acid (DNA) cassette containing the pullulanase gene from *B. deramificans* and the chloramphenicol acetyltransferase (*cat*) gene from *B. licheniformis*. The *cat* gene was used as a selectable marker in the transformation of the host strain. The transformed host strain was further modified by deletion of genes that encode two major endogenous proteases. The strain was subsequently subjected to a gene amplification procedure to increase the number of pullulanase gene copies. The final production strain was designated as BMP139. The strain is genetically stable and does not contain the plasmid DNA used in the transformation of the host strain.

1.2 Chemical and technical considerations

Pullulanase is produced by submerged, aerobic, pure culture fermentation of the genetically modified *B. licheniformis* production strain. The enzyme is secreted into the fermentation broth and is subsequently purified and concentrated. The enzyme concentrate is formulated with dextrose, sodium benzoate and potassium sorbate to produce a final product with the desired pullulanase activity and stability. Total organic solids (TOS) comprise several per cent of the final pullulanase preparation. The pullulanase enzyme preparation conforms to the General Specifications and Considerations for Enzyme Preparations Used in Food Processing (FAO, 2001).

The pullulanase enzyme preparation is used as a processing aid in the manufacture of corn syrups, including high-fructose corn syrup, potable alcohol and beer. In the production of sweeteners from starch, pullulanase is used in conjunction with glucoamylase to saccharify starch after its gelatinization with α-amylase. The action of these enzymes allows nearly complete (>95.5%) starch hydrolysis to monomeric glucose. The pullulanase enzyme preparation is typically used at levels ranging between 0.1 and 0.5 litre per tonne of starch on a dry weight basis.

In a typical brewing application, the recommended dosage of the pullulanase enzyme preparation is 0.5–2.0 kg per tonne of grist and up to 2 g per 100 litres during fermentation. In the production of potable alcohol, the pullulanase enzyme preparation is used at a rate of 0.2–0.3 kg per tonne of grist during saccharification and at a rate of 0.15 kg per tonne of grist during fermentation.

Pullulanase would be inactivated and/or removed during processing or purification of the reaction products, and its carryover to food is expected to be negligible.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

No information was available on the absorption, distribution, metabolism or excretion of pullulanase or on its effects on enzymes and other biochemical parameters.

Pullulanase has been evaluated for potential allergenicity using bioinformatics criteria recommended by the Codex Alimentarius Commission in its Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants (FAO/WHO, 2003). The amino acid sequence homology search between pullulanase and known allergens included in the Structural Database of Allergenic Proteins revealed no matches greater than 35% sequence identity within a sliding window of 80 amino acids. The search for exact matches of short amino acid fragments that could serve as potential linear immunoglobulin E (IgE) binding sites yielded two matches of seven amino acids with non-food allergens from the German cockroach and the mould *Penicillium citrinum*. These matches were further assessed by analysing the amino acid sequence of pullulanase for hydrophilicity. As neither of the matching amino acid sequences is strongly hydrophilic, they are not likely to be located on the surface of the intact pullulanase or to serve as IgE binding sites.

2.2 Toxicological studies

Toxicity data for the pullulanase preparation were provided by the sponsor or obtained from a Medline search using pullulanase in a keyword search. For the 13-week repeated-dose study and the genotoxicity studies, an ultrafiltrate pullulanase preparation, using a representative batch (Batch No. 3656), was produced according to the procedure used for commercial production, but was more concentrated. The liquid enzyme concentrate had an activity of 4251 acid-stable pullulanase units per gram (where an acid-stable unit is defined as the amount of pullulanase that liberates reducing sugars equivalent to 0.45 µmol of glucose per minute from pullulan at pH 5.0 and 40 °C) and a TOS value of 9.82% weight per weight (w/w). Stability testing indicated that thawed samples, which had a protein content of 69.8 mg/ml and a specific gravity of 1.034 g/ml, were stable at 4 °C for at least 7 days. For the studies reported by Modderman & Foley (1995), the ultrafiltrate was freeze-dried and had an activity of 1300 acid-stable pullulanase units per gram. The TOS value of this preparation was not reported.

2.2.1 Acute toxicity

No information was available on the acute toxicity of pullulanase.

2.2.2 Short-term studies of toxicity

In a study that conformed with good laboratory practice (GLP) requirements and the United States Food and Drug Administration's (USFDA) draft guidelines for subacute toxicity testing (USFDA, 1993), groups of 10 male and 10 female Crl:CD BR rats received diets containing a freeze-dried preparation of pullulanase at a

concentration of 0, 2000, 10 000 or 50 000 mg/kg for 28 days. The dose selection was based on the results of an earlier 2-week range-finding study in rats in which concentrations of pullulanase up to 50 000 mg/kg in the diet did not produce any adverse effects. The average daily dose in each group was calculated to be 0, 191, 950 and 4773 mg/kg body weight (bw) per day, respectively, in males and 0, 209, 1011 and 5153 mg/kg bw per day, respectively, in females. The experimental parameters determined were clinical signs, body weight, feed consumption, neurobehavioural testing (World Health Organization/International Programme on Chemical Safety [WHO/IPCS] functional observational battery), ophthalmic end-points, haematological parameters, clinical chemical end-points and urinalysis parameters, gross and microscopic appearance and organ weights. Blood for haematology and clinical chemistry was collected from five rats of each sex per group before dosing and then from all rats at necropsy. Ophthalmoscopy and urinalysis were performed for all rats before treatment and again just prior to the study termination. Neurobehavioural testing was performed during the last week of the study, and selected organs from all control and high-dose animals were weighed and prepared for microscopic evaluation. If any treatment-related adverse effects in tissues were observed at the high dose, then the same organs were examined at lower doses.

No treatment-related effects on mortality, clinical signs, body weight gain, feed consumption, neurobehavioural effects or ophthalmoscopy were observed. Statistically significant increases in mean and absolute thymus weights of males treated at 950 and 4773 mg/kg bw per day were considered to have no toxicological significance because similar organ weight changes were not observed in females and the increases were not associated with any histopathology.

Overall, it can be concluded that no toxicologically relevant effects were seen in this 28-day study of general toxicity in rats when pullulanase was administered in the diet at doses up to 4773 mg/kg bw per day and 5153 mg/kg bw per day in males and females, respectively. This dose, the highest dose tested, was therefore taken to be the no-observed-adverse-effect level (NOAEL) (Modderman & Foley, 1995).

In a study conducted in accordance with GLP requirements and largely to Organisation for Economic Co-operation and Development (OECD) Test Guideline 408, groups of 10 male and 10 female Sprague-Dawley (Crl:CD(SD)BR) rats received pullulanase (5 ml/kg bw) by oral gavage at a dose of 0, 625, 1250 or 2500 mg/kg bw per day for 13 weeks. Dose selection for the highest dose was based on achieving at least a 4900-fold excess over the 90th percentile mean human dietary exposure. The experimental parameters determined were clinical signs, body weight, feed consumption, neurobehavioural testing (WHO/IPCS functional observational battery), ophthalmic end-points, haematological parameters, clinical chemical end-points and urinalysis parameters, gross and microscopic appearance and organ weights. Ophthalmoscopy was performed for all rats before treatment and then prior to the study termination. Body weight and feed consumption were recorded weekly. Apart from neurobehavioural testing, which was completed for control and high-dose rats only during week 13, all other parameters were measured just prior to sacrifice. At necropsy, selected organs were removed, weighed and processed for histopathological examination. A microscopic examination was conducted on selected organs from control and high-dose animals.

No treatment-related effects on mortality, clinical signs, body weight gain, feed consumption, neurobehavioural effects or ophthalmoscopy were observed. Reductions in urinary sodium and calcium concentrations in females at 1250 and 2500 mg/kg bw per day achieved statistical significance after 13 weeks of treatment but were considered to have no toxicological significance because they were not observed in males and were not associated with any renal histopathology. In both sexes, organ weights, macroscropic pathology and histopathology were unaffected by treatment.

Overall, it can be concluded that the NOAEL was 2500 mg/kg bw per day (i.e. 246 mg TOS per kilogram body weight per day), the highest dose tested in this study (Boyer, Blouin & Jolette, 1999).

2.2.3 Long-term studies of toxicity and carcinogenicity

No information was available from long-term studies of the toxicity and carcinogenicity of pullulanase.

2.2.4 Genotoxicity

The results of the in vitro and in vivo genotoxicity studies with pullulanase (Batch No. 3656) are summarized in Table 1. The studies reported by Modderman & Foley (1995) conformed with GLP requirements and the USFDA's draft guidelines for genotoxicity (USFDA, 1993). All other reported studies were compliant with GLP and quality assurance requirements and appeared to conform with the principles of the various OECD test guidelines for genotoxicity testing.

2.2.5 Reproductive and developmental toxicity

No multigeneration or developmental toxicity studies on pullulanase were available.

2.3 Observations in humans

No information was available on the effects of pullulanase on humans.

3. DIETARY EXPOSURE

3.1 Introduction

Pullulanase is used as a processing aid in the manufacture of modified starches, in the production of high-fructose corn syrup and for the production of potable alcohol and beer. The Committee evaluated one submission received from a manufacturer of the enzyme preparation. Pullulanases have been regulated for use in Brazil, Canada, China, Denmark, France, Japan, Mexico and the Republic of Korea and are listed as generally recognized as safe in the USA. The Committee noted that the use of pullulanase from *Bacillus deramificans* expressed in *Bacillus licheniformis* would be substitutional for pullulanase from other sources and would not result in an increase in dietary exposure to the enzyme. However, as the TOS from this preparation could be different from those from other sources, a complete analysis of dietary exposure was performed.

Table 1. Genotoxicity of pullulanase in vitro and in vivo

End-point	Test system	Concentration	Result	Reference
In vitro				
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535, TA1537 and TA1538	Ten dose levels up to 5000 µg/plate, ±S9	Negative	Modderman & Foley (1995)
	S. typhimurium TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2uvrA	100–5000 µg/plate, ±S9	Negative	Wagner & Walton (1999)
Forward mutation	L5178Y mouse lymphoma	1000, 4000, 4400 or 5000 µg/ml, –S9 4000, 4200, 4600, 4800 or 5000 µg/ml, +S9	Negative	Modderman & Foley (1995)
Chromosomal aberration	Human lymphocytes	4 h incubation: 25–500 µg/ml, ±S9 20 h incubation: 7–200 µg/ml, –S9	Negative	Gudi & Schadly (1999)
In vivo				
Micronucleus formation/ chromosomal aberration	Swiss-Webster mouse	500, 889,1581, 2811 or 5000 mg/kg bw	Negative	Modderman & Foley (1995)

S9, 9000 × *g* supernatant from rat liver

Although it is expected that the enzyme would be inactivated and residues of the enzyme preparation would be removed in the production of final food products, an upper-bound exposure assessment approach was used to estimate dietary exposure. This approach employed the maximizing assumptions that 100% of the food products listed above would be manufactured using the pullulanase enzyme preparation and that 100% of the enzyme preparation would remain in the final food products. Additionally, it was assumed that the pullulanase enzyme preparation contains as much total protein and TOS as the "ultrafiltrate concentrate" used in the mutagenicity and 90-day oral studies. This test material enzyme preparation is a more concentrated version of the food production enzyme preparation, but without the formulation ingredients.

The Committee concluded that the tiered approach to assessing dietary exposure to pullulanase was not necessary, as a conservative upper estimate of dietary exposure was evaluated.

3.2 Exposure assessment

The two broad food uses of the pullulanase enzyme preparation were considered individually in order to arrive at a cumulative dietary exposure. Food consumption data from the USA were used. The Committee considered that

because consumption of high-fructose corn syrup is much higher in the USA than in the rest of the world, this estimate would be sufficient to encompass use scenarios for pullulanase.

3.2.1 High-fructose corn syrup production

The estimated per capita annual consumption of corn-based syrups and sweeteners in 2008 in the USA, based on the information provided by the United States Department of Agriculture (USDA), was 31.4 kg, which equates to approximately 85 g/day. For a 60 kg individual, consumption of these sweeteners would be less than 1.5 g/kg bw per day.

The pullulanase enzyme preparation is typically used at a concentration of 0.1–0.5 litre per tonne of starch. Assuming that it is used at 0.5 litre per tonne, the concentration of pullulanase enzyme preparation in corn-based syrups and sweeteners would be 0.565 mg/g (0.5 litre pullulanase per tonne dry starch × 1.13 g syrup per millilitre). The dietary exposure to the pullulanase enzyme preparation would be approximately 1 mg/kg bw per day (1.5 g corn-based syrup and sweeteners per kilogram body weight per day × 0.565 mg/g).

The daily intake of pullulanase enzyme preparation, expressed as TOS, would be 0.1 mg TOS per kilogram body weight per day (1 mg enzyme product per kilogram body weight per day × 9.82% TOS), assuming that the pullulanase enzyme preparation contains as much TOS as the ultrafiltrate concentrate.

3.2.2 Beer and spirits production

Based on the data compiled by the USDA for the year 2008, the annual per capita intake of beer in the USA was 82 litres, equivalent to a daily intake of 225 ml (http://www.ers.usda.gov/data/foodconsumption/spreadsheets/beverage.xls).

For mash brewing, the typical use concentration for pullulanase enzyme preparation is 0.5–2.0 kg per tonne of grist, with a maximum rate of 2 g per 100 litres (or 20 g per tonne) during fermentation. Therefore, the maximum concentration of pullulanase enzyme preparation used in the brewing process is 2.02 g enzyme product per kilogram of grist (2.0 g/kg grist + 20 mg/kg wort = 2.020 g enzyme product per kilogram).

Approximately 17 kg of grist are needed to make 100 litres of beer. Hence, 0.35 g enzyme product per litre of beer is obtained from grist (2.02 g enzyme product per kilogram × 17 kg = 34.34 g enzyme product per 100 litres).

Assuming that the pullulanase is not inactivated during the brewing process and that the enzyme preparation contains as much TOS as the ultrafiltrate concentrate (i.e. 9.82%), the maximum concentration of pullulanase (expressed as TOS) in beer would be 0.034 g/l of beer (0.35 g enzyme product per litre × 9.82% TOS). The daily exposure to pullulanase (expressed as TOS) from the consumption of beer would be 0.008 g/day (0.034 g TOS per litre × 0.225 litre per day). This is equivalent to 0.0001 g TOS per kilogram body weight per day for a 60 kg individual.

Based on data compiled by the USDA for the year 2008, the annual per capita intake of spirits in the USA was 5.3 litres, equivalent to a daily intake of 14.5 ml.

Using 60 kg as the default body weight, the daily consumption of spirits is 0.24 ml/kg bw per day (http://www.ers.usda.gov/data/foodconsumption/spreadsheets/beverage.xls).

In the production of potable alcohol, pullulanase enzyme preparation is added during saccharification (the breakdown of complex carbohydrates to fermentable simple sugars) at 0.2–0.3 kg per tonne of grist and during the fermentation at 0.15 kg per tonne of grist. Assuming a concentration of 0.3 kg per tonne for pullulanase enzyme preparation, the overall concentration used during the production of potable alcohol is 0.45 kg/tonne grist (0.3 kg/tonne grist + 0.15 kg/tonne grist). As the estimated yield of potable alcohol from 1 tonne of grist (mash) is 650 litres, 0.7 g of the enzyme preparation would be present in 1 litre of potable alcohol (0.45 kg enzyme product per tonne of grist = 0.45 kg enzyme product per 650 litres of potable alcohol), assuming no removal during distillation.

Assuming that pullulanase is not inactivated during the alcohol production process and that the enzyme preparation contains as much TOS as the ultrafiltrate concentrate (i.e. 9.82%), the maximum concentration of pullulanase (expressed as TOS) per litre of potable alcohol would be 0.07 mg (0.7 g enzyme product per litre × 9.82%). The daily exposure to pullulanase (expressed as TOS) through consumption of potable alcohol is approximately 0.000 02 mg/kg bw per day (0.07 mg TOS per litre × 0.24 ml/kg bw per day).

Combining the estimates from each of the pullulanase use processes results in a dietary exposure to TOS from the enzyme preparation of approximately 0.1 mg/kg bw per day. The Committee noted that the dietary exposure to pullulanase enzyme preparation is dominated by the contribution from the consumption of high-fructose corn syrup and considered this estimate to be sufficiently conservative for the purpose of completing a safety assessment for uses globally.

4. COMMENTS

4.1 Assessment of potential allergenicity

Pullulanase has been evaluated for potential allergenicity using bioinformatics criteria recommended by the Codex Alimentarius Commission in its Guideline for the Conduct of Food Safety Assessment of Foods Derived from Recombinant-DNA Plants.

The amino acid sequence homology search between pullulanase and known allergens included in the Structural Database of Allergenic Proteins revealed no matches greater than 35% sequence identity within a sliding window of 80 amino acids. The search for exact matches of short amino acid fragments that could serve as potential linear IgE binding sites yielded two matches of seven amino acids with non-food allergens from the German cockroach and the mould *Penicillium citrinum*. These matches were further assessed by analysing the amino acid sequence of pullulanase for hydrophilicity. As neither of the matching amino acid sequences is strongly hydrophilic, they are not likely to be located on the surface of the intact pullulanase or to serve as IgE binding sites.

The Committee concluded that pullulanase has no characteristics of any known allergen.

4.2 Toxicological data

For the 13-week repeated-dose oral toxicity study and the genotoxicity studies, an ultrafiltrate pullulanase preparation, using a representative batch (Batch No. 3656), was produced according to the procedure used for commercial production, but was more concentrated. The liquid enzyme concentrate had an activity of 4251 acid-stable pullulanase units per gram (where an acid-stable unit is defined as the amount of pullulanase that liberates reducing sugars equivalent to 0.45 µmol of glucose per minute from pullulan at a pH of 5.0 and a temperature of 40 °C) and a TOS value of 9.82% w/w. Stability testing indicated that thawed samples, which had a protein content of 69.8 mg/ml and a specific gravity of 1.034 g/ml, were stable at 4 °C for at least 7 days.

In studies of general toxicity in rats, no significant treatment-related effects were seen when the pullulanase enzyme was administered in the diet at doses of up to approximately 5000 mg/kg bw per day (no TOS level reported) for 28 days or 2500 mg/kg bw per day (i.e. 246 mg/kg bw per day as TOS) by gavage for 13 weeks. Therefore, the NOAEL was identified as 246 mg/kg bw per day as TOS, the highest dose tested in the 13-week study. Pullulanase enzyme was not mutagenic in an assay for mutagenicity in bacteria in vitro and was not clastogenic in an assay for chromosomal aberration in mammalian cells in vitro. Similarly, an assay for micronucleus formation in mice showed no evidence of a clastogenic effect in vivo.

4.3 Assessment of dietary exposure

Pullulanase is not expected to remain in food. However, an estimate of the theoretical dietary exposure to pullulanase was made by the Committee based on the level of TOS in the enzyme preparation and its uses in the saccharification of starch (high-fructose corn syrup production) and the production of potable alcohol and beer. The potential mean dietary exposure to pullulanase enzyme preparation based on national food consumption data for the adult population, assuming a body weight of 60 kg and a level of TOS similar to that in the tested material (9.82%), was 0.1 mg/kg bw per day as TOS. This estimate is conservative, as it is made assuming that all high-fructose corn syrup production and potable alcohol and beer production processes would use this enzyme preparation.

5. EVALUATION

A comparison of the conservative exposure estimates with the NOAEL of 246 mg/kg bw per day as TOS from the 13-week study of oral toxicity provides a margin of exposure of about 2500. The Committee established an acceptable daily intake (ADI) “not specified” for pullulanase from *B. deramificans* expressed in *B. licheniformis*, when used in the applications specified and in accordance with good manufacturing practice.

6. REFERENCES

Boyer M, Blouin P, Jolette J (1999). *A 13-week oral gavage toxicity study of GC797 (pullulanase) in the albino rat*. Unpublished report, ClinTrials BioResearch Ltd, Senneville, Quebec, Canada. Submitted to WHO by Genencor International, Palo Alto, CA, USA.

FAO (2001). *General specifications and considerations for enzyme preparations used in food processing*. Rome, Italy, Food and Agriculture Organization of the United Nations (http://www.fao.org/ag/agn/jecfa-additives/docs/enzymes_en.htm).

FAO/WHO (2003). *Guideline for the conduct of food safety assessment of foods derived from recombinant-DNA plants. CAC/GL 45-2003.* Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (http://www.codexalimentarius.net/download/standards/10021/CXG_045e.pdf).

FAO/WHO (2010). *Report of the Forty-second Session of the Codex Committee on Food Additives, Beijing, China, 15–19 March 2010*. Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (ALINORM 10/33/12; http://www.codexalimentarius.net/web/archives.jsp?year=10).

Gudi R, Schadly EH (1999). *In vitro mammalian chromosome aberration test.* Unpublished report, BioReliance, Rockville, MD, USA. Submitted to WHO by Genencor International, Palo Alto, CA, USA.

Modderman JP, Foley HH (1995). Safety evaluation of pullulanase enzyme preparation derived from *Bacillus licheniformis* containing the pullulanase gene from *Bacillus deramificans*. *Regulatory Toxicology and Pharmacology*, 21:375–381.

USFDA (1993). *Toxicological principles for the safety assessment of direct food additives and color additives used in food ("Red Book II")* [draft]. Washington, DC, USA, Food and Drug Administration.

Wagner VO, Walton EW (1999). *Bacterial reverse mutation assay with an independent repeat assay. Effects of dietary acrylamide on systematic oxidative stress and inflammatory markers in rats.* Unpublished report, BioReliance, Rockville, MD, USA. Submitted to WHO by Genencor International, Palo Alto, CA, USA.

QUINOLINE YELLOW (addendum)

First draft prepared by

U. Mueller,[1] M. DiNovi,[2] J.-C. Leblanc[3] and E. Vavasour[4]

[1] Food Standards Australia New Zealand, Canberra, Australia
[2] Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, Maryland, United States of America (USA)
[3] L'Agence nationale de la sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), Maisons-Alfort, France
[4] Ottawa, Ontario, Canada

1. EXPLANATION

Quinoline Yellow is a synthetic food colour. It is prepared by sulfonating either 2-(2-quinolyl)-1,3-indandione (unmethylated variety) or a mixture containing about two thirds 2-(2-quinolyl)-1,3-indandione and one third 2-[2-(6-methyl-quinolyl)]1,3-indandione (methylated variety). It consists essentially of sodium salts of a mixture

of disulfonates, monosulfonates and trisulfonates of the above compounds and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Quinoline Yellow was evaluated by the Committee at its present meeting at the request of the Codex Committee on Food Additives at its Forty-second Session (FAO/WHO, 2010). Toxicological data related to Quinoline Yellow were previously evaluated by the Committee at its eighth, thirteenth, eighteenth, twenty-second, twenty-fifth and twenty-eighth meetings (Annex 1, references *8*, *19*, *35*, *47*, *56* and *66*). At its eighth meeting, the Committee did not establish an acceptable daily intake (ADI) for Quinoline Yellow because of inadequate toxicological data. At its thirteenth meeting, the Committee reviewed the available data and established a temporary ADI of 0–1 mg/kg body weight (bw) based on a no-observed-effect level (NOEL)[1] of 500 mg/kg bw per day in a long-term feeding study in rats. The ADI was made temporary because of data gaps. In particular, the Committee noted the absence of suitable information on the metabolism and kinetics of Quinoline Yellow and a long-term feeding study in a second mammalian species. At its eighteenth meeting, the Committee considered a suitable long-term feeding study in rats. Using the results of that study, the Committee established a temporary ADI of 0–0.5 mg/kg bw based on the absence of any adverse effects at the highest tested dose of 50 mg/kg bw per day. The Committee reiterated its desire to review a three-generation reproduction study that was in progress, more information on metabolism and a long-term feeding study in a non-rodent species.

At its nineteenth meeting in 1975 (Annex 1, reference *38*), the Committee was informed that there were two types of Quinoline Yellow: non-methylated Quinoline Yellow and partially (30%) methylated Quinoline Yellow. The Committee indicated that data generated using either source could be used to define the toxicological hazard associated with Quinoline Yellow. At its twenty-second meeting, the Committee reviewed a three-generation reproduction study in rats but did not amend the temporary ADI. At its twenty-fifth meeting, the Committee was advised that two major studies were nearing completion and decided to extend the temporary ADI that it had established at its eighteenth meeting until the twenty-eighth meeting.

At the twenty-eighth meeting, the Committee reviewed new data on metabolism and a long-term repeated-dose study in mice that had been exposed to Quinoline Yellow in utero and through lactation. The Committee established an ADI of 0–10 mg/kg bw based on a NOEL of 10 000 mg/kg in the diet (equivalent to a range of 1000–1500 mg/kg bw per day) in the long-term study in mice.

At its present meeting, the Committee based its evaluation on data previously reviewed together with published information that had become available since the twenty-eighth meeting. No new unpublished toxicological studies were submitted following a public call for data. The Committee took note of the content of a recently completed review of Quinoline Yellow by the European Food Safety Authority (EFSA).

---

[1] At its sixty-eighth meeting (Annex 1, reference *187*), the Committee decided to differentiate between no-observed-effect level (NOEL) and no-observed-adverse-effect level (NOAEL). This NOEL would now be considered a NOAEL.

2. BIOLOGICAL DATA

2.1 Biochemical aspects

No new information was available on the absorption, distribution, metabolism and excretion of Quinoline Yellow or on its effects on enzymes and other biochemical parameters.

2.2 Toxicological studies

2.2.1 Acute toxicity

No new information was available on the acute toxicity of Quinoline Yellow.

2.2.2 Short-term studies of toxicity

No new information was available from short-term studies of the toxicity of Quinoline Yellow.

2.2.3 Long-term studies of toxicity and carcinogenicity

Two additional unpublished long-term chronic toxicity and carcinogenicity studies with a reproductive toxicity phase, carried out in the rat and in the mouse by Biodynamics Laboratories Inc. in 1980–1981, were summarized by SCCNFP (2004). These two studies were not included in the previous evaluation by the Committee.

In the first study, groups of mice (60 of each sex) were fed Quinoline Yellow at dietary levels of up to 50 000 mg/kg (equivalent to approximately 7500 mg/kg bw per day) for 23–24 months. No adverse toxic effects were observed, and no evidence of carcinogenicity was noted (SCCNFP, 2004).

In the second study, to assess the effects of chronic daily exposure to Quinoline Yellow in albino (CD) rats (60 of each sex per group), Quinoline Yellow was admixed in the diet at 0, 300, 1000, 5000 or 20 000 mg/kg (equivalent to 0, 15, 50, 250 and 1000 mg/kg bw per day, respectively) and fed ad libitum for 30 months. To permit in utero exposure, F_0 parental rats were fed prior to and subsequent to mating. After parturition and weaning, the F_1 pups were maintained on diets containing the same levels of Quinoline Yellow as administered to the parental generation. A second dietary exposure study (70 of each sex per group) with Quinoline Yellow at a concentration of 50 000 mg/kg in the diet (equivalent to 2500 mg/kg bw per day) was initiated after the United States Food and Drug Administration (USFDA) concluded that the 20 000 mg/kg diet level in the first study did not achieve the maximum tolerated dose (MTD). After parturition and weaning, the F_1 pups were maintained on diets containing the same levels of Quinoline Yellow as administered to the parental generation. The reproductive aspects of this study are described under section 2.2.5. For the chronic phase, offspring (70 of each sex) were selected randomly from each of the treated and control groups. Tissues from rats were prepared and sectioned for histopathological examination.

Lower body weights compared with controls were observed at Quinoline Yellow dietary levels of 20 000 and 50 000 mg/kg. The weights of the kidneys,

adrenals, spleen, thyroid, uterus and ovaries were reduced in the absence of any histopathological lesions at the same dose levels. No treatment-related effects were described at Quinoline Yellow dietary levels of 5000 mg/kg, equivalent to 250 mg/kg bw per day (SCCNFP, 2004). According to SCCNFP (2004), the USFDA derived a no-observed-adverse-effect level (NOAEL) of 1000 mg/kg bw per day from this study. However, in the absence of the original study data, the Committee was unable to independently verify the effects of Quinoline Yellow at 20 000 mg/kg diet on body weight. The effects of in utero exposure on reproductive toxicity are reported under section 2.2.5.

2.2.4 Genotoxicity

The genotoxicity of Quinoline Yellow is summarized in Table 1.

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration study

To assess the effects of chronic daily exposure to Quinoline Yellow in albino (CD) rats (60 of each sex per group), Quinoline Yellow was admixed in the diet at 0, 300, 1000, 5000 or 20 000 mg/kg (equivalent to 0, 15, 50, 250 and 1000 mg/kg bw per day, respectively) and fed ad libitum for 30 months in an unpublished study conducted by Biodynamics Laboratories Inc. in the early 1980s and reviewed by SCCNFP (2004). To permit in utero exposure, F_0 parental rats were fed for 2 months prior to mating and then continuously thereafter. A second dietary exposure study (70 of each sex per group) with Quinoline Yellow at 50 000 mg/kg diet (equivalent to 2500 mg/kg bw per day) was initiated after the USFDA concluded that the 20 000 mg/kg dietary level in the first study did not achieve the MTD. After parturition and weaning, the F_1 pups were maintained on diets containing the same levels of Quinoline Yellow as administered to the parental generation.

The pups of the F_0 dams were reported to have reduced survival coupled with lower weight gains during lactation at Quinoline Yellow dose levels of 5000 mg/kg in the diet (equivalent to 250 mg/kg bw per day) and above, although no other treatment-related effects on reproductive parameters were noted. The NOAEL for this study is considered to be 50 mg/kg bw per day, based on the available summarized information reported in SCCNFP (2004).

(b) Developmental toxicity

No information on the developmental toxicity of Quinoline Yellow was available.

2.3 Observations in humans

2.3.1 Case–control studies

Common clinical signs attributed to food intolerance often involve recurrent urticaria/angio-oedema, functional upper and/or lower gastrointestinal disturbances or nonspecific symptoms such as headache, nausea and lassitude. However, many

Table 1. Genotoxicity of Quinoline Yellow

End-point	Test system	Concentration	Result	Reference
In vitro				
Forward mutation	Mouse lymphoma L5178Y cells, $tk^{+/-}$ locus	118–3800 µg/ml, ±S9	Negative	Wollny (2000)
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA1535 and TA1537 and *Escherichia coli* WP2uvrA	33–5000 µg/plate, ±S9	Negative	Wollny (1999)
	S. typhimurium TA98, TA100, TA1535, TA1537 and TA1538	Up to 500 µg/ml (spot test method), ±S9	Negative	Blevins & Taylor (1982)
In vivo				
Micronucleus	NMRI mouse (5/sex)	500, 1000 or 2000 mg/kg bw	Negative	Honarvar (2003)

S9, 9000 × *g* supernatant from rat liver

of the reports on food colour intolerance are characterized by poorly controlled challenge procedures (Juhlin, 1981). Studies performed under properly controlled conditions imply that intolerance to food additives in patients with chronic urticaria/angio-oedema is uncommon (Supramaniam & Warner, 1986; Simon, 2003). The true prevalence estimates range around 0.03–2% (Weber et al., 1979; Hannuksela & Haahtela, 1987; Young et al., 1987; Fuglsang, 1994).

2.3.2 Clinical trials

The hypothesis that ingestion of mixtures of certain food colours and sodium benzoate increases the hyperactive behaviour of children was investigated using a community-based, double-blind, placebo-controlled, randomized crossover food challenge in which two groups of children aged 3 (n = 153) and 8 or 9 years (n = 144) received one of two mixtures of four food colour additives and sodium benzoate in a fruit drink administered at home by a parent. The children were self-identified from the general population and represented a range of behaviour from normal to hyperactive. All of the food colour additives except Quinoline Yellow were azo dyes. The food additives comprising mixture A (Sunset Yellow, Carmoisine, Tartrazine and Ponceau 4R in unequal proportions, plus sodium benzoate) and mixture B (Sunset Yellow, Carmoisine, Quinoline Yellow and Allura Red in equal proportions, plus sodium benzoate) reflected a mixture considered representative for sweets as they are consumed by children in the United Kingdom. On a body weight basis, the total dose of colour additives received by the 3-year-old children was 1.33 mg/kg bw per day from mixture A and 2.0 mg/kg bw per day from mixture B. For the 8- or 9-year-old children, the total dose was 0.8 mg/kg bw per day from mixture A and 2.0 mg/kg bw per day from mixture B. For sodium benzoate, the younger age group received a dose of 3 mg/kg bw per day from each mixture, whereas the older children received only 1.45 mg/kg bw per day. Behaviour was assessed through a novel global hyperactivity aggregate (GHA) measure, which comprised

an unweighted aggregate of standardized scores from validated attention deficit hyperactivity disorder (ADHD) behaviour assessment tools. Behaviour at home was assessed by parents and in school by teachers and independent observers for both age groups. An additional computer-based tool was used to assess behaviour for the 8- to 9-year-old group. A high GHA score indicated greater hyperactivity.

Ingestion of the fruit drink with mixture A, but not mixture B, significantly increased GHA scores for all 3-year-old children relative to the placebo control GHA scores and for the high-consumption subsets (high-consumption subsets consist of children who had consumed ≥85% of the drinks in each treatment week). For the 8- and 9-year-olds, a significant increase in GHA scores was not observed in either the entire sample or the high-consumption subset with mixture A relative to placebo, whereas significant increases in the entire group and the high-consumption subset were observed for mixture B. The magnitudes of the changes in GHA scores associated with the active challenges were small, with the effect sizes averaging about 0.18. This is approximately equivalent to less than a 10% difference between children with ADHD and children without that disorder. Variability in the results may have been introduced by the nearly 2-fold difference in doses of colour additives received by the 3-year-old children compared with the 8- and 9-year-old children and the 2-fold difference in the dose of colour additives received by the 8- and 9-year-old children consuming mixture A compared with mixture B. In addition, inconsistency in the timing of treatment relative to the observation of behaviour could have introduced variability in the context of the comment by the study authors that onset of hyperactive behaviour in response to food additives can be produced within 1 hour of consumption (McCann et al., 2007).

In order to investigate the hypothesis that the children's behaviour reported in the McCann et al. (2007) study was influenced by allelic variation in a number of genes that have previously been implicated in ADHD (Thapar et al., 1999; Swanson et al., 2000; Kuntsi & Stevenson, 2001), buccal swabs were collected for genotypic analyses of cellular deoxyribonucleic acid (DNA). The genes studied included genes from the dopamine (dopamine transporter [DAT1], dopamine D4 receptor [DRD4] and catechol *O*-methyl-transferase [COMT]), adrenergic (adrenergic receptor alpha 2A [ADRA2A]) and histamine (histamine *N*-methyl-transferase [HNMT]) neurotransmitter systems. The genotype analysis involved the detection of single nucleotide polymorphisms (two in HNMT, one in COMT, one in DRD4 and one in ADRA2A) in the genes. There was evidence that the HNMT T939C and the DRD4 4rs740373 polymorphisms correlated to the overall GHA score in the 3-year-old children. However, there was no significant relationship of the polymorphisms to the GHA scores in the 8- and 9-year-olds (Stevenson et al., 2010).

3. DIETARY EXPOSURE

3.1 Introduction

The Committee has not previously evaluated dietary exposure estimates for Quinoline Yellow. The Committee received a submission from EFSA concerning dietary exposure to Quinoline Yellow that was a part of its re-evaluation of the

safety of a number of artificial colours (EFSA, 2009). Additionally, the Committee accessed and considered the dietary exposure sections of a 2008 report from Food Standards Australia New Zealand (FSANZ) on artificial colours (FSANZ, 2008).

3.1.1 Food uses

Quinoline Yellow is used to colour both solid foods and beverages. In the European Union (EU), its use is permitted at the maximum levels shown in Table 2. Under the Australia New Zealand Food Code, it is permitted at levels up to 70 mg/kg in beverages and 290 mg/kg in other foods.

3.2 International estimates of dietary exposure

The Committee concluded that international estimates of dietary exposure to Quinoline Yellow made using Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diet information would not be appropriate, as Quinoline Yellow is always used at low levels in highly processed foods.

3.3 National estimates of dietary exposure

3.3.1 European Food Safety Authority

The 2009 EFSA report on the re-evaluation of Quinoline Yellow (E 104) as a food additive contained a thorough examination of dietary exposure to this colour. The analysis used a tiered approach, beginning with a budget screening method and continuing with additional refined estimates.

(a) Budget method

EFSA used a budget method (tier 1 approach) as described in the report of the Scientific Cooperation (SCOOP) Task 4.2 (EC, 1998). The generalized equation for the budget method is shown below.

EFSA assumed that the maximum permitted use levels considered were 200 mg/l for beverages and 500 mg/kg for solid foods. The default proportion (25%) of beverages and solid food that could contain the additive was considered adequate. Thus, a typical adult weighing 60 kg might consume 1.5 litres of coloured beverages and 375 g of coloured solid foods containing Quinoline Yellow, daily. The theoretical maximum daily exposure for adults would be:

(200 mg/l beverage × 0.1 litre beverage/kg bw × 0.25) + (500 mg/kg food × 0.025 kg food/kg bw × 0.25) = 5 + 3.125 = 8.1 mg/kg bw per day

A similar calculation was carried out for children assuming that the maximum level in beverages was 100 mg/l (after exclusion of alcoholic drinks). It was further assumed that 100% of beverages consumed could be coloured. The theoretical maximum daily exposure for children would be:

(100 mg/l beverage × 0.1 litre beverage/kg bw × 1) + (500 mg/kg food × 0.025 kg food/kg bw × 0.25) = 10 + 3.125 = 13.1 mg/kg bw per day

Table 2. Maximum permitted use levels of Quinoline Yellow in beverages and foodstuffs in the EU

Beverages	Maximum permitted level (mg/l)
Non-alcoholic flavoured drinks Americano Bitter soda, bitter vino Liquid food supplements/dietary integrators	100
Spirituous beverages Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails Fruit wines, cider and perry	200
Foodstuffs	**Maximum permitted level (mg/kg)**
Complete formulae for weight control intended to replace total daily food intake or an individual meal Complete formulae and nutritional supplements for use under medical supervision Soups	50
Flavoured processed cheese Fish paste and crustacean paste Smoked fish Savoury snack products and savoury coated nuts Meat and fish analogues based on vegetable proteins Jam, jellies and marmalades and other similar fruit preparations including low-calorie products	100
Edible ices Desserts including flavoured milk products	150
Fine bakery wares Candied fruit and vegetables, mostarda di frutta Preserves of red fruits Extruded or expanded savoury snack products	200
Pre-cooked crustaceans	250
Confectionery Mustard Fish roe Solid food supplements/dietary integrators	300
Decorations and coatings Sauces, seasonings, pickles, relishes, chutney and piccalilli Salmon substitutes Surimi	500
Edible cheese rind and edible casings	Quantum satis

(b) Refined estimates

Exposure estimates for children 1–10 years of age were performed based on detailed individual food consumption data from eight European countries (Belgium, Czech Republic, Finland, France, Germany, Italy, the Netherlands and Spain). Estimates for United Kingdom children aged 1.5–4.5 years were made using detailed individual food consumption data from the United Kingdom National Diet and Nutrition Survey (1992–1993) and with maximum permitted levels of use as specified in EU Directive 94/36/EC on food colours (EU, 1994) (tier 2 approach). The United Kingdom population was considered as representative of all EU adults for the Quinoline Yellow exposure estimates, as it was considered to be the population with the highest consumption of soft drinks in Europe. Additionally, the adult food consumption data for the United Kingdom population were considered to be more refined than those available from the EFSA Concise European Food Consumption Database.

The mean dietary exposure estimates for European children aged 1–10 years and weighing 25–30 kg when considering maximum permitted levels of use ranged from 0.8 to 3.5 mg/kg bw per day, whereas those at the 95th percentile were from 1.8 to 9.6 mg/kg bw per day. For United Kingdom children aged 1.5–4.5 years and weighing 15 kg, the mean dietary exposure was 3.1 mg/kg bw per day, and dietary exposure at the 97.5th percentile[1] was 7.3 mg/kg bw per day. Estimates reported for the United Kingdom adult population were 0.9 mg/kg bw per day at the mean and 2.1 mg/kg bw per day at the 97.5th percentile. For adults, the main contributors to the total anticipated exposure to Quinoline Yellow (>10% in all countries) were soft drinks (13–41%), fine bakery wares (e.g. viennoiserie, biscuits, cakes, wafers) (14–29%) and desserts (including flavoured milk products) (17–62%). Sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli accounted for 10–50% of exposure in four countries. Confectionery accounted for 11% of exposure in one country.

The tier 3 approach employed by EFSA used maximum reported Quinoline Yellow use levels in place of the maximum permitted levels of tier 2. In some, but not all, cases, these were lower than the levels used in tier 2. In this analysis, the dietary exposures to Quinoline Yellow for European children ranged from 0.45 to 2.0 mg/kg bw per day at the mean and from 1.1 to 4.1 mg/kg bw per day at the 95th percentile. For United Kingdom children aged 1.5–4.5 years, the mean dietary exposure was 1.8 mg/kg bw per day, and dietary exposure at the 97.5th percentile was 4.3 mg/kg bw per day. Estimates for the United Kingdom adult population were 0.5 mg/kg bw per day at the mean and 1.2 mg/kg bw per day at the 97.5th percentile. The main contributors to the total anticipated exposure to Quinoline Yellow (>10% in all countries) were soft drinks (10–39%), fine bakery wares (e.g. viennoiserie, biscuits, cakes, wafers) (14–60%) and desserts (including flavoured milk products) (14–57%). Confectionery accounted for 13–18% of exposure in two countries, and

[1] The United Kingdom 97.5th percentile estimates herein are made from the 97.5th percentile estimate from beverages combined with the per capita estimates from all other coloured foods.

Table 3. EFSA dietary exposure estimates for Quinoline Yellow

	Exposure (mg/kg bw per day)		
	Adults	Children 1.5–4.5 years old	Children 1–10 years old
Budget method	8.1		13.1[a]
Maximum permitted levels			
- Mean exposure	0.9	3.1	0.8–3.5
- Exposure at the 95th or 97.5th percentile	2.1	7.3	1.8–9.6
Maximum reported use levels			
- Mean exposure	0.5	1.8	0.45–2.0
- Exposure at the 95th or 97.5th percentile	1.2	4.3	1.1–4.1

[a] For children (age range not specified).

surimi, sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli accounted for 15% of exposure in one country.

The results of the EFSA tiered approach analyses are summarized in Table 3.

3.3.2 Food Standards Australia New Zealand

FSANZ included Quinoline Yellow in an overall survey of artificial colour use in foods in 2006. The foods and beverages examined were confectionery, ice cream, cheese, yoghurt, margarine, flavoured milk, flavoured soya beverages, soft drinks, cordials, fruit drinks, alcoholic drinks, biscuits, cakes, pastries, savoury snacks, breakfast cereals, pre-prepared meals, processed meats, sauces, toppings, jams/conserves and jelly. A small number of products that claimed to contain “no added colours” or “no artificial colour” were also sampled.

Assessments of dietary exposure to Quinoline Yellow were made for the Australian population aged 2 years and above, children aged 2–5 years, children aged 6–12 years, adolescents aged 13–18 years, adults aged 19–24 years and adults aged 25 years and above. The dietary exposures were estimated by combining usual patterns of food consumption, as derived from the 1995 National Nutrition Survey, with analysed levels of the colour in foods. Estimates were made using two scenarios: the mean colours scenario and the maximum colours scenario.

In the mean colours scenario, mean analytical concentrations of Quinoline Yellow in survey foods were used. Both detected and “non-detect” results were used to derive the mean analytical concentrations. It was assumed that the use of mean food colour concentrations represents the most realistic exposure for consumers of a range of brands and varieties of particular foods over a period of time. In the maximum colours scenario, estimates were made by using the maximum analytical

Table 4. FSANZ dietary exposures to Quinoline Yellow using the mean colours scenario

Population group	Mean exposure		90th percentile exposure	
	mg/person per day	mg/kg bw per day	mg/person per day	mg/kg bw per day
2–5 years old	0.05	<0.01	0.15	0.01
6–12 years old	0.06	<0.01	0.14	0.01
13–18 years old	0.08	<0.01	0.15	<0.01
19–24 years old	0.15	<0.01	0.38	0.01
25+ years old	0.17	<0.01	0.48	0.01
2+ years old	0.13	<0.01	0.36	0.01

concentrations of Quinoline Yellow in the survey foods. The use of maximum food colour concentrations assumed that every processed food consumed contained the highest concentration of each colour detected in the survey, in this case, Quinoline Yellow. The report states that this model will significantly overestimate exposure to added colours, except where products containing food colours at the highest levels of use are consumed every day. The estimates made using the maximum colours scenario were not used by FSANZ in its overall evaluation of the safety of the use of artificial colours.

For the Australian population aged 2 years and older, the mean dietary exposure to Quinoline Yellow was 0.13 mg/day, with a 90th percentile exposure of 0.36 mg/day. The highest subpopulation mean was 0.17 mg/day, for those 25+ years of age. The highest subpopulation 90th percentile exposure was 0.48 mg/day, also for those 25+ years of age. The highest estimates made using the maximum colours scenario were 0.41 mg/day at the mean (for those 25+ years of age and older) and 1.10 mg/day at the 90th percentile (for the 19- to 24-year-old subpopulation). The main contributors to dietary exposure were cakes, muffins and pastries, and sweet biscuits.

These results are summarized in Table 4.

3.4 Conclusions

The estimates of dietary exposure to Quinoline Yellow calculated by EFSA were much higher than those of FSANZ. The Committee concluded that this was due to EFSA's use of maximum permitted and maximum reported use levels in its tier 2 and tier 3 approaches, as opposed to FSANZ's use of the mean analysed levels for all foods. The latter approach is considered to be more realistic for preparing lifetime dietary exposure estimates. The Committee concluded that 4 mg/kg bw per day, the tier 3, 97.5th percentile EFSA estimate for children 1–10 years of age, should be considered for use in the safety assessment for Quinoline Yellow, as it represents the most conservative assessment. However, it recognized that the FSANZ estimate for children, 0.01 mg/kg bw per day, was a more realistic

dietary exposure estimate because of the extensive post-market analyses used in its preparation.

4. COMMENTS

4.1 Toxicological data

This summary of the available toxicological data combines the studies previously reviewed (Annex 1, references *8*, *19*, *35*, *47*, *56* and *66*) with recently published data.

The absorption of ingested Quinoline Yellow is between 3% and 4% in rats and dogs, with most being excreted unchanged in faeces. There is evidence that some of the absorbed Quinoline Yellow is excreted in bile. Quinoline Yellow does not accumulate in tissues, and 85–90% of the Quinoline Yellow absorbed from the gastrointestinal tract is excreted unchanged in the urine.

Repeated-dose feeding studies for 90 days in the rat showed an absence of adverse effects at dose levels up to 2500 mg/kg bw per day. Two-year feeding studies confirmed the absence of any treatment-related effects in mice, rats and dogs at doses equivalent to 1500, 500 and 50 mg/kg bw per day, respectively. The long-term feeding studies in rodents also indicated that Quinoline Yellow was not carcinogenic. This was consistent with an absence of any genotoxicity reported previously or in the new studies completed since the Committee last considered Quinoline Yellow.

No adverse effects on reproductive performance in rats over three generations were reported following dietary exposure to Quinoline Yellow at the highest tested dose of 50 mg/kg bw per day. Similarly, a comprehensive two-generation study involving 65 rats of each sex per group on test showed no adverse reproductive effects at Quinoline Yellow concentrations up to 10 000 mg/kg in the diet (equivalent to a dose range of 1000–1500 mg/kg bw per day).

There are reports suggesting that asthma or chronic idiopathic urticaria/angio-oedema in humans may be induced by oral exposure to Quinoline Yellow. However, most of these reports are characterized by poorly controlled challenge procedures. Although recent studies performed with better control conditions are available, no conclusion on idiosyncratic responses to Quinoline Yellow could be drawn from the available evidence.

The administration of six different food colours and a preservative, sodium benzoate, and the presence of multiple methodological deficiencies limited the value of a recent study that investigated a possible relationship between hyperactivity in children and the consumption of beverages containing food colours. The use of mixtures in dosing studies does not permit any observed effects to be ascribed to individual components.

4.2 Assessment of dietary exposure

Estimates of dietary exposure to Quinoline Yellow prepared and published by EFSA and FSANZ were available to the Committee. The estimates of dietary

exposure to Quinoline Yellow calculated by EFSA were much higher than those of FSANZ (0.01 mg/kg bw per day for children at the 90th percentile). The Committee concluded that this was due to the use of maximum reported use levels by EFSA, as opposed to the use of the mean analysed levels for all foods by FSANZ. The latter approach was considered by the Committee to be more realistic for preparing long-term dietary exposure estimates. Because of the conservative assumptions used by EFSA in making the exposure estimates, the Committee concluded that EFSA's 97.5th percentile estimate of 4 mg/kg bw per day for children should be considered in the safety assessment for Quinoline Yellow in addition to the more realistic FSANZ estimate.

5. EVALUATION

The Committee noted that there were no new data submitted that would provide a suitable basis on which to revise the existing ADI of 0–10 mg/kg bw for Quinoline Yellow. However, the Committee was aware of unpublished long-term studies in mice and rats with in utero exposure to Quinoline Yellow that had been completed by Biodynamics Laboratories in 1980–1981, but had not been submitted for evaluation. One of these studies was used by EFSA to establish its ADI for Quinoline Yellow. As the results of these studies in rodents might affect the existing ADI, the Committee established a temporary ADI of 0–5 mg/kg bw, incorporating an additional 2-fold uncertainty factor, pending submission of the Biodynamics Laboratories studies by the end of 2013. The previously established ADI of 0–10 mg/kg bw was withdrawn. The conservative exposure estimates were below the upper limit of the temporary ADI.

6. REFERENCES

Blevins RD, Taylor DE (1982). Mutagenicity screening of twenty five cosmetic ingredients with the *Salmonella*/microsome test. *Journal of Environmental Science and Health. Part A, Toxic/Hazardous Substances and Environmental Engineering*, 17:217–239.

EC (1998). *Report on methodologies for the monitoring of food additive intake across the European Union. Final report submitted by the Task Coordinator, 16 January 1998.* Reports of a Working Group on Scientific Cooperation on Questions Relating to Food, Task 4.2. Brussels, Belgium, European Commission, Directorate General III Industry (SCOOP/INT/REPORT/2).

EFSA (2009). Scientific opinion on the re-evaluation of Quinoline Yellow (E 104) as a food additive. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). *The EFSA [European Food Safety Authority] Journal*, 7(11):1329 [40 pp.] (http://www.efsa.europa.eu/en/scdocs/doc/1329.pdf; accessed 29 May 2011).

EU (1994). European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. *Official Journal of the European Communities*, L 237:13–29.

FAO/WHO (2010). *Report of the Forty-second Session of the Codex Committee on Food Additives, Beijing, China, 15–19 March 2010.* Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (ALINORM 10/33/12; http://www.codexalimentarius.net/web/archives.jsp?year=10).

FSANZ (2008). *Survey of added colours in foods available in Australia. Study of concentrations in foods including dietary exposure assessment and risk characterisation.* Food Standards

Australia New Zealand (http://www.foodstandards.gov.au/_srcfiles/Colours%20Survey_Final%20Report%2022%20Oct%2008%20_2_.pdf; accessed 29 May 2011).

Fuglsang G et al. (1994). Adverse reactions to food additives in children with atopic symptoms. *Allergy*, 49:31–37.

Hannuksela M, Haahtela T (1987). Hypersensitivity reactions to food additives. *Allergy*, 42:561–575.

Honarvar N (2003). *Micronucleus assay in bone marrow cells of the mouse with D&C Yellow 10*. Rossdorf, Germany, RCC-CCR (Test Report No. 741301) [cited in SCCNFP, 2004].

Juhlin L (1981). Recurrent urticaria: clinical investigation of 330 patients. *British Journal of Dermatology*, 104:369–381.

Kuntsi J, Stevenson J (2001). Psychological mechanisms in hyperactivity: II. The role of genetic factors. *Journal of Child Psychology and Psychiatry*, 42:211–219.

McCann D et al. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomized, double-blinded, placebo-controlled trial. *Lancet*, 370:1560–1567.

SCCNFP (2004). *Opinion of the Scientific Committee on Consumer Products and Non-Food Products Intended for Consumers Concerning Acid Yellow 3, Colipa No. C54* (SCCNFP/0789/04; http://ec.europa.eu/health/archive/ph_risk/committees/sccp/documents/out276_en.pdf; accessed 29 May 2011).

Simon RA (2003). Adverse reactions to food additives. *Current Allergy and Asthma Reports*, 3:62–66.

Stevenson J et al. (2010). The role of histamine degradation gene polymorphisms in moderating the effects of food additives on children's ADHD symptoms. *American Journal of Psychiatry*, 167:1108–1115.

Supramaniam G, Warner JO (1986). Artificial food additive intolerance in patients with angio-oedema and urticaria. *Lancet*, 2:907–909.

Swanson JM et al. (2000). Dopamine genes and ADHD. *Neuroscience and Biobehavioural Reviews*, 24:21–25.

Thapar A et al. (1999). Genetic basis of attention deficit and hyperactivity. *British Journal of Psychiatry*, 174:105–111.

Weber RW et al. (1979). Incidence of bronchoconstriction due to aspirin, azo dyes, non-azo dyes, and preservatives in a population of perennial asthmatics. *Journal of Allergy and Clinical Immunology*, 64:32–37.

Wollny HE (1999). Salmonella typhimurium *and* Escherichia coli *reverse mutation assay with D&C Yellow 10 (C.I. 47005).* Rossdorf, Germany, RCC-CCR Project 636301 [cited in SCCNFP, 2004].

Wollny HE (2000). *Cell mutation assay at the thymidine kinase locus (TK +/–) in mouse lymphoma L5178Y cells with D&C Yellow 10 (C.I. 47005).* Rossdorf, Germany, RCC-CCR Project 636302 [cited in SCCNFP, 2004].

Young E et al. (1987). The prevalence of reaction to food additives in a survey population. *Journal of the Royal College of Physicians*, 21:241–247.

SUNSET YELLOW FCF (addendum)

First draft prepared by

U. Mueller,[1] M. DiNovi,[2] J.-C. Leblanc[3] and E. Vavasour[4]

[1] Food Standards Australia New Zealand, Canberra, Australia
[2] Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, Maryland, United States of America (USA)
[3] L'Agence nationale de la sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), Maisons-Alfort, France
[4] Ottawa, Ontario, Canada

1. EXPLANATION

Sunset Yellow FCF (Chemical Abstracts Service No. 2783-94-0) is a synthetic food colour. It is also known as Orange Yellow S, CI Food Yellow 3, FD&C Yellow 6 and C.I. 15985. Sunset Yellow FCF consists principally of the disodium salt of 6-hydroxy-5-(4-sulfonatophenylazo)-2-naphthalenesulfonic acid and subsidiary colouring matters together with sodium chloride and/or sodium sulfate as the principal uncoloured components.

Sunset Yellow FCF was evaluated by the Committee at its present meeting at the request of the Codex Committee on Food Additives at its Forty-second Session (FAO/WHO, 2010). The Committee was asked to evaluate all data necessary for the assessment of the safety, dietary exposure and specifications for Sunset Yellow FCF. Sunset Yellow FCF was evaluated by the Committee at its eighth and twenty-sixth meetings (Annex 1, references *8* and *59*). At its eighth meeting, the Committee considered that sufficient toxicological data were available to establish an acceptable daily intake (ADI) of 0–5 mg/kg body weight (bw) for Sunset Yellow FCF. At the twenty-sixth meeting, the Committee considered new studies on long-term and reproductive toxicity and established an ADI of 0–2.5 mg/kg bw.

At its present meeting, the Committee based its evaluation on data previously reviewed together with published information that had become available since Sunset Yellow FCF was last considered by the Committee. There were no new unpublished toxicological studies submitted following a public call for data. However, a comprehensive review of one unpublished long-term feeding study in mice and two in rats was provided by the United States Food and Drug Administration (USFDA). The Committee also took note of the content of a recently completed review of Sunset Yellow FCF by the European Food Safety Authority (EFSA).

2. BIOLOGICAL DATA

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

No new information was available on the absorption, distribution and excretion of Sunset Yellow FCF.

2.1.2 Biotransformation

In vitro studies comparing Sunset Yellow FCF reduction rates between bacterial suspensions derived from rat intestines and those derived from human faeces indicated that the rate was approximately 4–5 times greater in rats. There was very little difference in activity between the five tested male human faecal samples in spite of a considerable divergence in age, daily diets and living circumstances (Watabe et al., 1980).

Oral gavage administration of Sunset Yellow FCF at 200 and 1000 mg/kg bw to two groups of nine male Swiss mice showed that nearly all was recovered in the faeces within 24 hours. The main aromatic amine metabolite, sulfanilic acid,

was also present. The bacterial metabolic transformation rates appeared to be dependent on the administered dose level, with the biotransformation rate being higher at 200 mg/kg bw than at 1000 mg/kg bw (Poul et al., 2009).

2.1.3 Effects on enzymes and other biochemical parameters

To investigate the inhibition of the activities of human phenolsulfotransferase-P (PST-P), phenolsulfotransferase-M (PST-M) and monoamine oxidase A and B by eight food colours, including Sunset Yellow FCF and Ponceau 4R, each colour was tested separately at a concentration of 1, 5 or 25 µmol/l using conventional in vitro testing protocols. The substrates used for the enzymes were phenol for PST-P, tyramine for PST-M and [^{14}C]tyramine for both monoamine oxidases. At a concentration of 25 µmol/l, Sunset Yellow FCF and Ponceau 4R completely inhibited PST-P activity. However, at 5 and 1 µmol/l, the extent of inhibition was 55% and 17%, respectively, for Sunset Yellow FCF and 39% and 11%, respectively, for Ponceau 4R. Sunset Yellow FCF and Ponceau 4R had little to no inhibitory effect on PST-M or monoamine oxidase activities at a concentration of 25 µmol/l (Gibb, Glover & Sandler, 1987).

Kuno & Mizutani (2005) investigated the influence of Sunset Yellow FCF on the activities of phase I and phase II drug-metabolizing enzymes (cytochrome P450 [CYP] 2A6, uridine diphosphate glucuronosyltransferase [UGT] 1A6 and 2B7) derived from bovine liver microsomes. Their findings indicated that Sunset Yellow FCF is neither a substrate nor an inhibitor of the enzymes studied.

Osman et al. (2004) investigated the inhibitory effect of Sunset Yellow FCF and its major metabolite sulfanilic acid on cholinesterase activity in plasma and erythrocytes. For the in vivo study, Sunset Yellow FCF or sulfanilic acid was administered to groups of five male albino rats in the diet at 400 mg/kg for an unspecified duration. This concentration in the feed gave a final dose of 4 mg/kg bw per day, assuming a reported daily feed intake of only 2 g/day for 200 g rats, which seems remarkably low for ad libitum feed consumption (FAO/WHO, 2009). The usual feed consumption for a 200 g rat is around 10 times this value (i.e. 20 g/day). For a feed intake of 20 g/day, the dose for each rat would increase to 40 mg/kg bw per day.

Even though Sunset Yellow FCF and sulfanilic acid inhibited the acetylcholinesterase activity in rat erythrocytes by 14% and 31%, respectively, no clinical signs in rats were reported. In contrast, the cholinesterase activity in plasma was reduced by 23% and 13% for Sunset Yellow FCF and sulfanilic acid, respectively. It is known that rat plasma contains approximately equal proportions of acetylcholinesterase and butyrylcholinesterase, whereas human plasma contains almost exclusively one form of cholinesterase—namely, butyrylcholinesterase (García-Ayllón et al., 2006).

In a separate in vitro study, the cholinesterase inhibitory effect of Sunset Yellow FCF on human blood from 10 male volunteers was investigated; a 50% inhibition of activity required a concentration of 0.33 mmol/l (149 mg/l) in plasma and 0.24 mmol/l (108 mg/l) in erythrocytes. Similar results for cholinesterase inhibition in human plasma in vitro were reported by Osman et al. (2002). For sulfanilic acid,

a concentration of 0.77 mmol/l resulted in a modest enzyme activity reduction of 13% and 14% for plasma and erythrocytes, respectively. Given the uncertainty in the administered dose in rats and the high concentrations of Sunset Yellow FCF needed to elicit appreciable inhibition of cholinesterase activity in vitro, the effect on physiological cholinesterase activity levels is unlikely to contribute much to its hazard profile.

2.2 Toxicological studies

2.2.1 Acute toxicity

No new information was available on the acute toxicity of Sunset Yellow FCF.

2.2.2 Short-term studies of toxicity

(a) Mouse

Sunset Yellow FCF (FD&C Yellow No. 6; purity 92%) was administered in the diet to groups of 10 male and 10 female B6C3F1 mice at 0, 6000, 12 500, 25 000, 50 000 or 100 000 mg/kg for 12 weeks followed by 1 week of recovery with control diet only. Dose selection was based on an earlier 14-day repeated-dose dietary study that revealed no deaths or signs of toxicity in groups of five mice of each sex. In the main study, mice were observed twice per day and weighed weekly. Gross and histopathological examinations were performed on all animals.

Mean body weight gain was reduced by more than 10% among male mice at the 100 000 mg/kg dietary intake level and in females at all concentration levels tested in a dose-related manner from 12 500 to 100 000 mg/kg diet. Gross and histopathological examinations revealed no treatment-related lesions in male or female mice at any intake level. The concentrations selected for the chronic study were 12 500 and 25 000 mg/kg diet (NCI/NTP, 1982).

(b) Rat

Sunset Yellow FCF (FD&C Yellow No. 6; purity 92%) was administered to groups of 10 male and 10 female F344 rats at a concentration of 0, 6000, 12 500, 25 000, 50 000 or 100 000 mg/kg in the diet for 12 weeks followed by 1 week of control diet only. Dose selection was based on an earlier 14-day repeated-dose dietary study that revealed no deaths or signs of toxicity in groups of five of each sex. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed weekly. Gross and histopathological examinations were performed on all animals.

No animals died during the study. Reductions in mean body weight gain exceeding 9.8% were reported in male rats at intake levels of 25 000, 50 000 and 100 000 mg/kg. In female rats, similar reductions in mean body weight gain were reported at 12 500, 25 000, 50 000 and 100 000 mg/kg diet. Bone marrow hyperplasia was reported in all animals at 50 000 and 100 000 mg/kg diet. The

concentrations selected for the chronic study were 12 500 and 25 000 mg/kg diet (NCI/NTP, 1982).

Two different mixtures containing Tartrazine, Brilliant Blue FCF, Sunset Yellow FCF and Carmoisine in undefined, but different, ratios (mixtures A and B; purity not specified) were administered in their diet to six groups of 10 male albino rats. Each colour mixture was purchased from a local market and was added to the rat diet at a concentration aimed to achieve a daily dose of 800 mg/kg bw. The dosing duration was 30 days, 60 days or 60 days with a 30-day recovery period for each mixture separately. A number of haematological and clinical chemistry parameters were measured, such as haemoglobin, red blood cell and white blood cell counts, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), total protein, albumin, globulin and albumin/globulin ratio, glucose, total lipids, triglycerides, cholesterol, cholesterol/high-density lipoprotein ratio, urea, creatinine, liver deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) concentration, thyroid hormones (triiodothyronine [T_3] and thyroxine [T_4]) and growth hormone. At the end of dosing, only kidney, liver and stomach sections were prepared for histopathological examination.

Effects considered to be treatment related because they increased in magnitude with duration and showed an apparent decline following the recovery period were elevated levels of serum total lipids, cholesterol, triglycerides, total protein, globulin and serum ALT. Haematological investigations demonstrated selective neutropenia and lymphocytosis in the absence of any significant changes in total white blood cell counts and significantly decreased haemoglobin concentrations and red blood cell counts. Eosinophilia was observed only in rats receiving mixture A. Histopathological examination revealed few adverse effects in the stomach. However, in the liver and kidneys, congested blood vessels and areas of haemorrhage were observed in rats receiving mixture B. A brown pigment deposition was observed in the portal tracts and Kupffer cells of the liver as well as in the interstitial tissue and renal tubular cells of the kidney. As there was no information available on either the purity or ratios of the various colours in the two mixtures, the relevance of these observations in defining the hazard of Sunset Yellow FCF is limited (Aboel-Zahab et al., 1997).

Sunset Yellow FCF (purity not specified) was administered orally by gavage to groups of 10 male albino rats. A 5 mg/kg bw dose was given daily for 30 days, after which five rats per group were killed, and the remaining five rats had a 2-week recovery period before sacrifice. Body weight was measured before and at the end of treatment. Clinical chemistry testing included AST, ALT, bilirubin, creatinine, urea, total protein, albumin, inorganic phosphorus and calcium. No necropsy or histopathology was undertaken.

Cage-side observations indicated that the Sunset Yellow FCF–treated rats appeared to be more aggressive, nervous and generally more active relative to controls. Small but statistically significant increases relative to controls were observed in AST activity (119% of control; $P < 0.05$) and ALT activity (113% of control; $P < 0.05$), indirect bilirubin (175% of control; $P < 0.05$) but not total bilirubin, and urea (132% of control; $P < 0.01$). Body weight (88% of control; $P < 0.01$), total protein (86% of control; $P < 0.05$) and serum globulin (67% of control; $P < 0.01$)

were significantly reduced. Apart from body weight (88% of control; $P < 0.01$), AST activity (114% of control; $P < 0.05$) and urea (111% of control; $P < 0.05$), all other levels returned to control levels at the end of the recovery period. The toxicological significance of these small magnitude changes in the very limited range of measured parameters is difficult to interpret. This, coupled with the very small number of test animals, limits the study's value in defining the hazard profile of Sunset Yellow FCF (Helal et al., 2000; Mekkawy et al., 2001).

Sunset Yellow FCF (purity not specified) in combination with sodium nitrate (143 mg/kg bw per day) was administered orally by gavage to groups of 10 male albino rats at 7 mg/kg bw per day. After daily dosing for 30 days, five rats per group were killed, and the remaining five were held for a 2-week recovery period before sacrifice. Body weight was measured before and at the end of treatment. Clinical chemistry testing included AST, ALT, calcium, glucose, cholesterol, T_3 and T_4, γ-glutamyl transferase (GGT), lactate dehydrogenase (LDH), creatine phosphokinase (CPK), AP, bilirubin, creatinine, urea, total protein, albumin, inorganic phosphorus and calcium. Haematology investigations included measuring haematocrit, haemoglobin concentration and white blood cell count. No necropsy or histopathology was undertaken.

Exposure to the mixture significantly decreased body weight, red blood cell count, haemoglobin, haematocrit, white blood cell count, serum inorganic phosphorus, serum protein and serum albumin. Significant increases were observed in serum glucose, T_3 and T_4, calcium, GGT, LDH, CPK, AP and cholesterol. After the 14-day recovery period, most biochemical and haematological parameters had recovered. In view of the presence of sodium nitrate in combination with Sunset Yellow FCF of undefined purity, it is not possible to ascertain the toxicological significance of these findings in defining the hazard of Sunset Yellow FCF (Helal, 2001).

Mathur et al. (2005a) purchased a yellow food colorant that was claimed to be Sunset Yellow FCF (purity not specified) from a local market in India and administered it to groups of 10 male Wistar rats in their diet at concentrations of 0, 5000 or 30 000 mg/kg (equivalent to 0, 250 and 1500 mg/kg bw per day) for 90 days. At sacrifice, only testes were collected, prepared and sectioned for light microscopy.

Histologically, the testes of the 5000 mg/kg diet group showed degenerative changes in some seminiferous tubules. Spermatogonia in the basal layer of seminiferous tubules were found to have an abnormal shape. Arrested maturation was observed in many tubules. Mature sperm were absent, but Leydig cells and Sertoli cells appeared to be normal. Testes of the rats treated with Sunset Yellow FCF at 30 000 mg/kg diet showed an increase in the degenerative changes. Necrotic areas appeared irregular, involving many tubules, and the affected tubules displayed extensive desquamation and sloughing off of almost all the seminiferous epithelium lining the basement membrane. Seminiferous tubules near the degenerated ones appeared normal. In most of the tubules, pycnotic spermatocytes at the germinal elements were seen. In some other tubules, pycnotic spermatogenesis was arrested at the spermatogonial or spermatocyte stage, whereas in a few tubules, transformation into spermatozoa could be seen. It was reported that Sertoli cells had virtually obliterated the lumen in some degenerating tubules, and they were highly

vacuolated. The Leydig cells and blood vessels appeared normal. The histological observations on testes revealed that almost 50% of the tubules displayed signs of toxicity. At both dose levels, the AP activity and cholesterol level in serum were significantly increased and the serum protein level was significantly decreased. The effect on serum AP activity amounted to +151% of control ($P < 0.001$) and +128% of control ($P < 0.001$) at the low and high dose levels, respectively.

As it is well known that an azo dye precursor such as 1,3-dinitrobenzene can induce testicular lesions very similar to those described in this study (Hess et al., 1988) and there is considerable uncertainty surrounding the identity and purity of the administered colorant, this study was considered to be unsuitable to contribute to the hazard characterization of Sunset Yellow FCF.

In another study, Mathur et al. (2005b) administered an uncharacterized sample of yellow food colorant, reported to be Sunset Yellow FCF, to male albino rats (10 per group) in their diet for 90 days at 0, 5000 or 30 000 mg/kg (equivalent to 0, 250 and 1500 mg/kg bw per day, respectively). The investigators reported significant and dose-related elevations in levels of total lipids and various lipid fractions. The maximum increase was seen in triglyceride levels, and the lowest increase was observed in cholesterol levels. The authors concluded that changes in lipid metabolism were caused by liver damage. However, given the uncertainties surrounding the identity and purity of the administered material, this study was considered to be unsuitable to contribute to the hazard characterization of Sunset Yellow FCF.

Another study that investigated the histological effects of a Sunset Yellow/Tartrazine mixture (no information on the ratio or purity reported) in Wistar rats (five of each sex per group) was reported by Ching et al. (2005). The colour mixture was orally administered by gavage to rats at doses of 500, 1000 or 2000 mg/kg bw on 3 consecutive days. Gross examination of tissues revealed marked ulcerative lesions and haemorrhage on the antra of stomachs of rats given the mixture at 2000 mg/kg bw, but only hyperaemia at 1000 mg/kg bw. At 1000 mg/kg bw, mild splenomegaly, hepatomegaly and enlarged pale kidneys were observed in the rats administered the colour mixture at 1000 or 2000 mg/kg bw by either oral or intraperitoneal injection. Histopathological examination of sections from the liver, kidney, spleen, stomach and ileum of rats treated with Sunset Yellow FCF revealed a variety of dose-related degenerative, inflammatory and proliferative lesions, which included necrosis, especially in the liver. Necrosis was observed in the kidneys, glomeruli and renal papillae as well as in splenic tissue. Given the uncertainties surrounding the identity and purity of the administered material and the small number of animals, this study was considered to be unsuitable to contribute to the hazard characterization of Sunset Yellow FCF.

2.2.3 Long-term studies of toxicity and carcinogenicity

(a) Mouse

Sunset Yellow FCF (FD&C Yellow No. 6; purity 92%) was administered to groups of 50 male and 50 female B6C3F1 mice at a concentration of 0, 12 500 (only 49 males per group) or 25 000 mg/kg in the diet for 103 weeks, and then

the animals were placed on a control diet for 1 week. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed at least monthly. Gross and histopathological examinations were performed on all animals. Tissues examined included skin (abdominal), lungs and bronchi, trachea, bone, bone marrow (femur), thigh muscle, spleen, lymph nodes, thymus, heart, salivary glands, liver, pancreas, oesophagus, stomach, duodenum, jejunum, ileum, caecum, colon, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, testis, prostate, mammary gland, uterus, ovary, brain, epididymis, eye and all tissue masses.

The mean body weights of male and female mice receiving Sunset Yellow FCF at a dietary concentration of 25 000 mg/kg were slightly lower (<10%) than those of the control animals throughout most of the study. However, the survival of male and female mice was similar between treated animals and controls (males: control 38/50 [76%], low dose 40/50 [80%] and high dose 33/50 [66%]; and females: control 38/50 [76%], low dose 35/50 [70%] and high dose 43/50 [86%]). An increased incidence of hepatocellular carcinomas was reported among males in the low-dose (46%) and high-dose (32%) groups relative to the control males (26%), but it achieved statistical significance ($P = 0.02$) only at the low dose. In contrast, no significant differences were observed in females. The increased incidence of hepatocellular carcinomas in male mice was not considered to be related to the test material because of the variability in tumour occurrence in control male B6C3F1 mice and because the incidence was not significantly increased in high-dose male mice. The investigators reported that under the conditions of the bioassay, there was no clear evidence of carcinogenicity of Sunset Yellow FCF in B6C3F1 mice at doses up to 25 000 mg/kg diet (equivalent to 3750 mg/kg bw per day) (Huff, 1982; NCI/NTP, 1982). Although there was a body weight decrement among males and females at the highest tested dose compared with control animals, it was less than 10%, a value not considered to be biologically significant; the no-observed-adverse-effect level (NOAEL) for this study is therefore considered to be 25 000 mg/kg diet (equivalent to 3750 mg/kg bw per day).

Sunset Yellow FCF (FD&C Yellow No. 6; purity 91%) was administered to CD-1 COBS (ICR-derived) mice ad libitum at a dietary level of 5000, 15 000 or 50 000 mg/kg. Two separate groups were fed the control diet. Each group consisted of 60 males and 60 females that were randomly assigned. The study was terminated at 20 months for the male mice and at 23 months for the female mice by sacrificing the surviving animals.

The mortality rate was higher in male mice fed diets containing Sunset Yellow FCF at a dietary concentration of 50 000 mg/kg than in the male control groups ($P < 0.01$, life table analysis). Mean body weights of male mice of this dosage group were less than those of the pooled controls throughout the study, despite elevated feed consumption. In female mice, elevated feed consumption occurred for the group fed Sunset Yellow FCF at 50 000 mg/kg in the diet. Elevated feed consumption also occurred for the male mice fed Sunset Yellow FCF at 5000 and 15 000 mg/kg diet.

Complete histopathology was done on the controls and the high-dose groups. Histopathological examinations of all tissue masses and other gross changes

of an uncertain nature were done for mice in the two lowest dose groups. Gross postmortem examinations of treated mice revealed yellow to orange discoloration of the gastrointestinal tract by Sunset Yellow FCF.

The gross and microscopic examinations of tissues and organs of mice from this study revealed no adverse morphological changes that could be attributed to treatment with Sunset Yellow FCF. A detailed statistical analysis indicated that this colour additive had no effect on the incidence of any tumour type or on the time to tumour formation at concentrations up to 50 000 mg/kg diet. However, based on deaths and a body weight decrement relative to the controls at 50 000 mg/kg diet, the NOAEL for the study is 15 000 mg/kg diet (equivalent to 2250 mg/kg bw per day) (USFDA, 1986).

(b) Rat

Sunset Yellow FCF (FD&C Yellow No. 6; purity 92%) was administered to groups of 50 male and 50 female F344 rats at a concentration of 0, 12 500 or 25 000 mg/kg in the diet for 103 weeks, followed by a control diet for 1 week. Ninety male and 90 female rats served as concurrent controls. Animals were housed five per cage and fed the test diet ad libitum. The animals were observed twice per day and weighed at least monthly. Gross and histopathological examinations were performed on all animals. Tissues examined were the same as those described for the mouse (see above).

The mean body weights of male rats at the highest concentration were slightly lower than those of the control animals throughout the study (<10%). The survival of male and female rats was similar between treated animals and controls (males: control 70/90 [78%], low dose 36/50 [72%] and high dose 38/50 [76%]; and females: control 66/88 [75%], low dose 40/50 [80%] and high dose 37/50 [74%]). Histopathological examination revealed no evidence of carcinogenicity related to treatment with the test material, and no other effects were reported. Therefore, under the conditions of the 2-year bioassay, there was no clear evidence of carcinogenicity of Sunset Yellow FCF in F344 rats at doses up to 25 000 mg/kg diet (equivalent to 1250 mg/kg bw per day) (Huff, 1982; NCI/NTP, 1982). As the body weight decrement among males at the highest dose was less than 10%, a value not considered to be biologically significant, the NOAEL for this study is considered to be 25 000 mg/kg diet (equivalent to 1250 mg/kg bw per day).

To assess the effects of chronic daily exposure to Sunset Yellow FCF (FD&C Yellow No. 6; purity 91%) in albino (CD) rats (60 of each sex per group), Sunset Yellow FCF was admixed in the diet at 7500, 15 000 or 30 000 mg/kg (equivalent to 375, 750 and 1500 mg/kg bw per day, respectively), and the rats were fed ad libitum for 2 years. To permit in utero exposure, F_0 parental rats were fed prior to and subsequent to mating. After parturition and weaning, the F_1 pups were maintained on diets containing the same levels of Sunset Yellow FCF as administered to the parental generation. Although the survival of rats was shortened and the delivered pups had lower body weights relative to controls at a Sunset Yellow FCF dietary concentration of 30 000 mg/kg, a second dietary exposure study with a Sunset Yellow FCF concentration of 50 000 mg/kg in diet (equivalent to 2500 mg/kg bw per day) was initiated after the USFDA concluded that the 30 000 mg/kg level in the

first study did not achieve the maximum tolerated dose (MTD) for the purpose of maximizing the potential to assess carcinogenicity. The reproductive aspects of this study are described under section 2.2.5. Two groups of controls were fed the plain diet in the first study, and one group of controls was included in the second study.

For the chronic phase, offspring (70 of each sex) were selected randomly from each of the treated and control groups. Interim sacrifice and necropsy of 10 rats of each sex per group were performed 1 year after the initiation of the long-term feeding study in the offspring. Tissues from all rats in the three control groups and the two high-dose groups (30 000 and 50 000 mg/kg diet) were prepared and sectioned for histopathological examination. Histopathological evaluations were performed on all tissue masses and gross lesions from animals in the two lowest dose groups.

Although feed consumption by all treated groups in both studies was generally increased, the body weights of rats fed the 50 000 mg/kg diet were lower compared with controls throughout the study. In the chronic phase, survival of F_0 males fed Sunset Yellow FCF at 50 000 mg/kg diet was decreased ($P = 0.01$). There were no treatment-related differences in haematology measurements, clinical chemistry measurements or urinalysis in either study. A slight increase in kidney weights in females treated with Sunset Yellow FCF at 50 000 mg/kg diet was observed at the 1-year interim sacrifice, but this was not associated with any histopathological abnormality. At the terminal sacrifice, females treated with Sunset Yellow FCF at dietary concentrations of 30 000 and 50 000 mg/kg had increased mean relative kidney weights ($P = 0.05$).

There was no evidence of a carcinogenic effect in male rats that could be attributed to treatment with Sunset Yellow FCF. However, in treated female rats, a higher incidence of adrenal medullary tumours was observed at Sunset Yellow FCF concentrations of 30 000 and 50 000 mg/kg diet. As a confirmatory step, the USFDA requested that additional histopathological sections be prepared from the preserved adrenal tissue blocks and subjected to scrutiny by a panel of pathologists. Adrenal glands from female rats of the two lowest dose groups not previously examined were also sectioned and submitted for review.

According to the USFDA panel of pathologists, the incidence of adrenal medullary tumours (phaeochromocytoma) in female rats was 12/68 (17.6%) in control 1A, 6/66 (9.1%) in control 1B, 7/66 (10.6%) in the 7500 mg/kg diet group, 9/64 (14.1%) in the 15 000 mg/kg diet group and 15/66 (22.7%) in the 30 000 mg/kg diet group. Prevalence statistical tests for high dose relative to combined controls and dose-related trend test yielded *P*-values of 0.054 and 0.022, respectively, for these incidences. (The prevalence analysis is a time-adjusted statistical test for comparing incidences of lesions considered to be non-lethal.) In the second study, the 50 000 mg/kg diet group and its control female group had medullary tumour incidences of 15/68 (22.1%) and 5/70 (7.1%), respectively. Utilizing the low control incidence in this study, the prevalence *P*-value for this comparison was 0.01.

The following considerations suggest that it is unlikely that a causal relationship exists between the occurrence of phaeochromocytoma and exposure to Sunset Yellow FCF:

- small magnitude of difference and absence of a dose–response relationship;
- absence of any precancerous lesions;
- morphological similarity of adrenal medullary lesions in treated and control rats;
- unaffected latency period (time to tumour onset);
- absence of a similar response in male rats;
- lack of concordance with other Sunset Yellow FCF carcinogenicity studies.

In the first rat study on Sunset Yellow FCF, no renal cortical tumours were reported in the high-dose group (30 000 mg/kg diet) or either of the two control groups of female rats. In the second study, five females in the 50 000 mg/kg diet group were reported to have a renal tubular adenoma. No renal cortical tumours were reported in the control groups of the first study, although one control female rat had a transitional cell tumour. In the second study, females in the group fed Sunset Yellow FCF at 50 000 mg/kg diet and the control group had a high incidence of chronic progressive nephrosis (also called old-rat nephropathy).

It is unlikely that these renal cortical tumours are related to Sunset Yellow FCF exposure because of a number of considerations, such as the following:

- A survey of more than 230 chemicals tested in chronic toxicity bioassays by the United States National Cancer Institute/National Toxicology Program showed that chemically induced neoplasia of the kidney occurred more commonly in males than in females (Kluwe et al., 1984). There is no reason to believe that Sunset Yellow FCF would be an exception to the general observation that male rats are more sensitive than female rats to the effects of renal cortical carcinogens.
- There were no malignant or precancerous renal tubular lesions observed in the female rats.
- If Sunset Yellow FCF were a carcinogen for rat kidneys, then at least some of the proliferative lesions observed in the study should have progressed to become malignant.
- There was no reduction in the latency period.
- There is an absence of corroborative evidence from other carcinogenicity studies.

Based on these considerations, it is considered unlikely that Sunset Yellow FCF is able to induce carcinogenic activity in rats at concentrations up to 50 000 mg/kg diet (equivalent to 2500 mg/kg bw per day). However, reduced body weight gain and poorer survival in rats were observed at a Sunset Yellow FCF dietary concentration of 50 000 mg/kg diet (equivalent to 2500 mg/kg bw per day). Hence, the NOAEL for the chronic phase of the study is 30 000 mg/kg diet (equivalent to 1500 mg/kg bw per day) (USFDA, 1986).

2.2.4 Genotoxicity

The genotoxicity of Sunset Yellow FCF is summarized in Table 1.

Table 1. Genotoxicity of Sunset Yellow FCF

End-point	Test system	Concentration	Result	Reference
In vitro				
Forward mutation	L61178Y tk^+/tk^- mouse lymphoma cells	Up to 5000 µg/ml, ±S9	Negative (–S9) Weak positive, LOED 1000 µg/ml (+S9)	McGregor et al. (1988)
Reverse mutation	*Salmonella typhimurium* TA98, TA100	Pooled bile (0.2, 0.4, 0.6 ml/plate; ±S9) from 5 rats, 4 h after treatment with 1500 mg/kg bw by oral gavage	Negative	Wever et al. (1989)
		Pooled urine (0.2, 0.4, 0.6 ml/plate; ±S9) from 6 rats, three doses of 1500 mg/kg bw over 2 days		
		Pooled faecal extracts (0.2, 0.4, 0.6 ml/plate; ±S9) from 6 rats, three doses of 1500 mg/kg bw over 2 days	Negative (–S9) Positive (+S9)	
	S. typhimurium TA98, TA100, TA1535, TA1537	10–250 mg/plate, ±S9	Negative	Muzzall & Cook (1979)
	S. typhimurium TA98, TA100, TA1535, TA1537, TA1538	Up to 5000 µg/plate, ±S9 Metabolites sulfanilic acid and 4-amino-1-naphthalenesulfonic acid: both up to 5000 µg/plate	Negative	Chung, Fulk & Andrews (1981)
	S. typhimurium TA92, TA94, TA98, TA100, TA1535, TA1537	Up to 5000 µg/plate, ±S9	Negative	Ishidate et al. (1984)
	S. typhimurium TA1535, TA1538; *Escherichia coli* WP2uvrA	Up to 10 000 µg/ml (liquid culture method), ±S9	Negative	Haveland-Smith & Combes (1980)

End-point	Test system	Concentration	Result	Reference
Chromosomal aberration	Chinese hamster fibroblast line	Up to 6000 µg/ml, –S9 24 h and 48 h incubation	Positive	Ishidate et al. (1984)
	Chinese hamster ovary cells	Up to 5000 µg/ml, ±S9 8 h incubation without S9 and 2 h with S9	Negative	Ivett et al. (1989)
		Up to 5000 µg/ml, ±S9 25 h incubation without S9 and 2 h with S9	Negative	
In vivo				
Unscheduled DNA synthesis	NMRI, C3H and C57B1 mouse; Chinese hamster (all by oral gavage)	500, 1000, 1500 or 2000 mg/kg bw and 30 h exposure	Negative	Wever et al. (1989)
Micronucleus formation	CRH mouse or hooded rat (oral gavage) bone marrow cells	500, 1000 or 2000 mg/kg bw and 24 h or 48 h exposure	Negative	Westmoreland & Gatehouse (1991)
	Swiss mouse (oral gavage); colonic epithelial cells	200 or 1000 mg/kg bw and 24 h exposure	Negative	Poul et al. (2009)
	Chinese hamster bone marrow cells	1500 mg/kg bw and 30 h exposure	Negative	Wever et al. (1989)
Comet assay	ddY mouse (oral gavage); glandular stomach, colon, liver, kidney, urinary bladder, lung, brain and bone marrow examined	2000 mg/kg bw and 3 h or 24 h exposure	Negative	Sasaki et al. (2002)

LOED, lowest-observed-effect dose; S9, 9000 × *g* supernatant from rat liver

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration study

To assess the effects of chronic daily exposure to Sunset Yellow FCF (FD&C Yellow No. 6; purity 91%) in albino (CD) rats (60 of each sex per group), Sunset Yellow FCF was admixed in the diet at 7500, 15 000 or 30 000 mg/kg diet (equivalent to 375, 750 and 1500 mg/kg bw per day, respectively), and the rats were fed ad libitum for 2 years. To permit in utero exposure, F_0 parental rats were fed prior to and subsequent to mating. After parturition and weaning, the F_1 pups were maintained on diets containing the same levels of Sunset Yellow FCF as administered to the parental generation. Although survival of the rats was shortened and delivered pups had lower body weights at the Sunset Yellow FCF concentration of 30 000 mg/kg diet relative to the control, a second dietary exposure study with Sunset Yellow FCF at a concentration of 50 000 mg/kg diet (equivalent to 2500 mg/kg bw per day) was initiated after the USFDA concluded that the 30 000 mg/kg dietary level in the first study did not achieve the MTD. Two groups of controls were fed the plain diet in the first study, and one group of controls was included in the second study.

Reduced pup weights occurred at 15 000, 30 000 and 50 000 mg/kg diet, and offspring survival was reduced in the groups treated with Sunset Yellow FCF at 30 000 or 50 000 mg/kg diet. The body weights of rats fed Sunset Yellow FCF at 50 000 mg/kg diet were lower throughout the study. However, the body weights of treated and control rats of the first study were generally comparable, despite lower pup weights in groups treated with Sunset Yellow FCF at 15 000 and 30 000 mg/kg diet relative to the controls. No other adverse effects were observed. Hence, the NOAEL for reproductive toxicity is 7500 mg/kg diet (equivalent to 375 mg/kg bw per day) (USFDA, 1986).

Sunset Yellow FCF (purity >85%) was administered to groups of Crj:CD-1 mice (10 of each sex) in their diet at a concentration of 0, 1500, 3000 or 6000 mg/kg diet in a two-generation reproduction study. Dosing commenced when mice in the F_0 generation were 5 weeks old and continued until mice in the F_1 generation were 9 weeks old. Feed consumption data indicated no significant difference between controls and those groups consuming Sunset Yellow FCF. The actual dose of Sunset Yellow FCF achieved in each group was approximately 250, 500 and 1000 mg/kg bw per day for non-lactating mice at dietary concentrations of 1500, 3000 and 6000 mg/kg diet, respectively. However, during lactation, the dose ranged up to approximately 890, 1650 and 3360 mg/kg bw per day, respectively. Mice were weighed on days 0, 2, 4, 7, 14, 21, 28 and 30 during the pre-mating phase. Females were paired 1:1 with males and separated after 5 days. Dams were allowed to deliver and rear their offspring in solitude. Pups were weighed on postnatal days (PNDs) 0, 4, 7, 14 and 21. Functional and behavioural parameters, such as surface righting (PNDs 4 and 7), negative geotaxis (body righting on an inclined plane; PNDs 4 and 7), cliff avoidance (PND 7), swimming behaviour (PNDs 4 and 14) and olfactory orientation (PND 14), were measured in all F_1 pups during PNDs 0–21. On PND 49, all pups performed in a multiple water T-maze daily for 3 consecutive days.

For F_1 pups, there were no significant differences observed in litter size, litter weight or sex ratio. During lactation, there were no dose-related changes in body weight or survival. Score frequencies for swimming direction at PND 4 (but not PND 14) were significantly depressed in both males and females, but were dose dependent only in females. The score for swimming head angle was also affected in a dose-dependent manner in females. Scores for surface righting at PND 7, but not at PND 4, and negative geotaxis at PND 4, but not at PND 7, were affected in males only at the middle dose (3000 mg/kg diet). These effects were not dose related. Several of the other measured functional and behavioural parameters differed from controls, but not in a manner that allowed the investigators to conclude that they were related to exposure to Sunset Yellow FCF. Hence, in the absence of any effects on the reproductive parameters and inconsistent neurological outcomes, it can be concluded that the NOAEL for this study is 6000 mg/kg in the diet (approximately 1000 mg/kg bw per day), the highest dose tested (Tanaka, 1996).

(b) Developmental toxicity

No information on the developmental toxicity of Sunset Yellow FCF was available.

2.3 Observations in humans

2.3.1 Case–control studies

Common clinical manifestations attributed to food intolerance usually include recurrent urticaria/angio-oedema, functional upper and/or lower gastrointestinal disturbances or nonspecific symptoms such as headache, nausea and lassitude. However, many of the reports on food colour intolerance are characterized by poorly controlled challenge procedures (Mikkelsen et al., 1978; Ibero et al., 1982; Schultz-Ehrenburg & Gilde, 1987; Wilson & Scott, 1989; Worm et al., 2000). Studies performed under properly controlled conditions imply that intolerance to food additives in patients with chronic urticaria/angio-oedema is uncommon (Supramaniam & Warner, 1986; Simon, 2003). The true prevalence estimates range around 0.03–2% (Hannuksela & Haahtela, 1987; Young et al., 1987; Fuglsang et al., 1994).

2.3.2 Clinical trials

In 1990, Pollock and Warner conducted a study to investigate claims by parents that the behaviour of their 39 children aged between 3 and 15 years (mean 8.9 years) improved on a synthetic food additive–free diet, but deteriorated markedly with lapses from the diet. The children were recruited for the study from a paediatric allergy clinic and from a population survey of food additive intolerance. The trial consisted of a double-blind, placebo-controlled challenge with synthetic food colours. The food colours tested were a mixture of Tartrazine (50 mg), Sunset Yellow FCF (25 mg), Carmoisine (25 mg) and Amaranth (25 mg). Only 19 children completed the double-blind, placebo-controlled challenge study with artificial food colours. In these children, food colours were shown to have an adverse effect on a daily Conners' rating of behaviour, but most parents could not detect these changes.

This disparity between the results of the behaviour scores and the parents' weekly assessments is important when it is remembered that entry into the study was based on the parents' claim to be able to detect when their children had consumed food additives. It is important to acknowledge that the doses of food colours used in this trial were considerably greater than the amounts the children are likely to consume through food. The authors postulated that a pharmacological mechanism of food additive intolerance—namely, histamine release from basophils—was possible.

Bateman et al. (2004) investigated the behavioural effects on 3-year-old children (n = 277) of ingesting a high-dose azo food dye mixture containing Sunset Yellow FCF, Tartrazine, Carmoisine and Ponceau 4R (5 mg of each) and sodium benzoate (45 mg) in a double-blind, placebo-controlled study. The children were classified as having hyperactivity (HA) (using two different activity scales: emotionality, activity and sociability; and Weiss–Werry–Peters) or not, with and without atopy (AT) (i.e. positive skin prick test with a number of known protein allergens), in a 2 × 2 group design (AT/HA, non-AT/HA, AT/non-HA, non-AT/non-HA). Over a 4-week period, the children received either the azo dye mixture with fruit juice or placebo (fruit juice only) on the 2nd and 4th weeks. Children's behaviour was assessed by research psychologists using validated tests and by the parents. Using assessments made by the parents, there were significant reductions in hyperactive behaviour during the withdrawal phase. Furthermore, there were significantly greater increases in hyperactive behaviour during the active period compared with the placebo period. These effects were not influenced by the presence or absence of previously diagnosed hyperactivity or by the presence or absence of atopy. However, there were no significant differences detected based on objective interactive testing by psychologists in the clinic.

A follow-up study was conducted to further investigate the association of ingestion of a mixture of food colour additives and sodium benzoate with hyperactive behaviour in children. The hypothesis was tested using a community-based, double-blind, placebo-controlled, randomized crossover food challenge in which two groups of children aged 3 years (n = 153) and 8 or 9 years (n = 144) received one of two mixtures of four food colour additives and sodium benzoate in a fruit drink administered at home by a parent. The children were self-identified from the general population and represented a range of behaviour from normal to hyperactive. All of the food colour additives except for Quinoline Yellow were azo dyes. The food additives comprising mixture A (Sunset Yellow, Carmoisine, Tartrazine and Ponceau 4R in unequal proportions plus sodium benzoate) were those tested in the Bateman et al. (2004) study, whereas mixture B (Sunset Yellow, Carmoisine, Quinoline Yellow and Allura Red in equal proportions plus sodium benzoate) reflected a mixture considered representative for sweets as they are consumed by children in the United Kingdom. On a body weight basis, the total dose of colour additives received by the 3-year-old children was 1.33 mg/kg bw per day from mixture A and 2.0 mg/kg bw per day from mixture B. For the 8- or 9-year-old children, the total dose was 0.8 mg/kg bw per day from mixture A and 2.0 mg/kg bw per day from mixture B. For sodium benzoate, the younger age group received a dose of 3 mg/kg bw per day from each mixture, whereas the older children received only 1.45 mg/kg bw per day. Behaviour was assessed through a novel

global hyperactivity aggregate (GHA) measure, which comprised an unweighted aggregate of standardized scores from validated attention deficit hyperactivity disorder (ADHD) behaviour assessment tools. Behaviour at home was assessed by parents and in school by teachers and independent observers for both age groups. An additional computer-based tool was used to assess behaviour for the 8- and 9-year-old group. A high GHA score indicated greater hyperactivity.

Ingestion of the fruit drink with mixture A, but not mixture B, significantly increased GHA scores for all 3-year-old children relative to the placebo control GHA scores and for the high-consumption subsets (high-consumption subsets consist of children who had consumed ≥85% of the drinks in each treatment week). For the 8- and 9-year-olds, a significant increase in GHA scores was not observed in either the entire sample or the high-consumption subset with mixture A relative to placebo, whereas significant increases in the entire group and the high-consumption subset were observed for mixture B. The magnitudes of the changes in GHA scores associated with the active challenges were small, with the effect sizes averaging about 0.18. This is approximately equivalent to less than a 10% difference between children with ADHD and children without that disorder. Variability in the results may have been introduced by the nearly 2-fold difference in doses of colour additives received by the 3-year-old children compared with the 8- and 9-year-old children and the 2-fold difference in the dose of colour additives received by the 8- and 9-year-old children consuming mixture A compared with mixture B. In addition, inconsistency in the timing of treatment relative to the observation of behaviour could have introduced variability in the context of the comment by the study authors that onset of hyperactive behaviour in response to food additives can be produced within 1 hour of consumption (McCann et al., 2007).

3. DIETARY EXPOSURE

3.1 Introduction

The Committee has not previously evaluated dietary exposure estimates for Sunset Yellow FCF. The Committee received a submission from EFSA concerning dietary exposure to Sunset Yellow FCF that was a part of its re-evaluation of the safety of a number of artificial colours (EFSA, 2009). Additionally, the Committee accessed and considered the dietary exposure sections of a 2008 report from Food Standards Australia New Zealand (FSANZ) on artificial colours (FSANZ, 2008).

3.1.1 Food uses

Sunset Yellow FCF is used to colour both solid foods and beverages. In the European Union (EU), its use is permitted at the maximum levels shown in Table 2. Under the Australia New Zealand Food Code, it is permitted at levels up to 70 mg/kg in beverages and 290 mg/kg in other foods.

3.2 International estimates of dietary exposure

The Committee concluded that international estimates of dietary exposure to Sunset Yellow FCF made using Global Environment Monitoring System – Food

Table 2. Maximum permitted use levels of Sunset Yellow FCF in beverages and foodstuffs in the EU

Beverages	Maximum permitted level (mg/l)
Non-alcoholic flavoured drinks	50
Bitter soda, bitter vino Liquid food supplements/dietary integrators	100
Spirituous beverages Aromatized wines, aromatized wine-based drinks and aromatized wine-product cocktails Fruit wines, cider and perry	200
Foodstuffs	**Maximum permitted level (mg/kg)**
Confectionery Fine bakery wares Edible ices Desserts including flavoured milk products Complete formulae for weight control intended to replace total daily food intake or an individual meal Complete formulae and nutritional supplements for use under medical supervision Soups	50
Flavoured processed cheese Fish paste and crustacean paste Smoked fish Savoury snack products and savoury coated nuts Meat and fish analogues based on vegetable proteins Jam, jellies and marmalades and other similar fruit preparations including low-calorie products	100
Sobrasada	135
Candied fruit and vegetables, mostarda di frutta Preserves of red fruits Extruded or expanded savoury snack products	200
Pre-cooked crustaceans	250
Mustard Fish roe Solid food supplements/dietary integrators	300
Decorations and coatings Sauces, seasonings, pickles, relishes, chutney and piccalilli Salmon substitutes Surimi	500
Edible cheese rind and edible casings	Quantum satis

Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diet information would not be appropriate, as Sunset Yellow FCF is always used at low levels in highly processed foods.

3.3 National estimates of dietary exposure

3.3.1 European Food Safety Authority

The 2009 EFSA report on the re-evaluation of Sunset Yellow FCF (E 110) as a food additive contained a thorough examination of dietary exposure to this colour. The analysis used a tiered approach, beginning with a budget screening method and continuing with additional refined estimates.

(a) Budget method

EFSA used a budget method (tier 1 approach) as described in the report of the Scientific Cooperation (SCOOP) Task 4.2 (EC, 1998). The generalized equation for the budget method is shown below.

EFSA assumed that the maximum permitted use levels considered were 200 mg/l for beverages and 500 mg/kg for solid foods. The default proportion (25%) of beverages and solid food that could contain the additive was considered adequate. Thus, a typical adult weighing 60 kg might consume 1.5 litres of coloured beverages and 375 g of coloured solid foods containing Sunset Yellow FCF, daily. The theoretical maximum daily exposure for adults would be:

(200 mg/l beverage × 0.1 litre beverage/kg bw × 0.25) + (500 mg/kg food × 0.025 kg food/kg bw × 0.25) = 5 + 3.125 = 8.1 mg/kg bw per day

A similar calculation was carried out for children, assuming that the maximum level in beverages was 50 mg/l (after exclusion of alcoholic drinks). It was further assumed that 100% of beverages consumed could be coloured. The theoretical maximum daily exposure for children would be:

(50 mg/l beverage × 0.1 litre beverage/kg bw × 1) + (500 mg/kg food × 0.025 kg food/kg bw × 0.25) = 5 + 3.125 = 8.1 mg/kg bw per day

(b) Refined estimates

Exposure estimates for children 1–10 years of age were performed based on detailed individual food consumption data from eight European countries (Belgium, Czech Republic, Finland, France, Germany, Italy, the Netherlands and Spain). Estimates for United Kingdom children aged 1.5–4.5 years were made using detailed individual food consumption data from the United Kingdom National Diet and Nutrition Survey (1992–1993) and with maximum permitted levels of use as specified in the EU Directive 94/36/EC on food colours (EU, 1994) (tier 2 approach). The United Kingdom population was considered as representative of all EU adults for the Sunset Yellow FCF exposure estimates, as it was considered to be the population with the highest consumption of soft drinks in Europe. Additionally, the

adult food consumption data for the United Kingdom population were considered to be more refined than those available from the EFSA Concise European Food Consumption Database.

The mean dietary exposure estimates for European children aged 1–10 years and weighing 25–30 kg when considering maximum permitted levels of use ranged from 0.3 to 2.5 mg/kg bw per day, whereas those at the 95th percentile were from 0.7 to 6.7 mg/kg bw per day. For United Kingdom children aged 1.5–4.5 years and weighing 15 kg, the mean dietary exposure was 1.4 mg/kg bw per day, and dietary exposure at the 97.5th percentile[1] was 3.5 mg/kg bw per day. Estimates reported for the United Kingdom adult population were 0.5 mg/kg bw per day at the mean and 1.1 mg/kg bw per day at the 97.5th percentile. For adults, the main contributors to the total anticipated exposure (>10%) were soft drinks (40%), sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli (14%) and fruit wines, cider and perry (13%).

The tier 3 approach employed by EFSA used maximum reported Sunset Yellow FCF use levels in place of the maximum permitted levels of tier 2. In some, but not all, cases, these were lower than the levels used in tier 2. In this analysis, the dietary exposures to Sunset Yellow FCF for European children ranged from 0.2 to 2.1 mg/kg bw per day at the mean and from 0.6 to 5.8 mg/kg bw per day at the 95th percentile. For United Kingdom children aged 1.5–4.5 years, the mean dietary exposure was 1.1 mg/kg bw per day, and dietary exposure at the 97.5th percentile was 3.2 mg/kg bw per day. Estimates for the United Kingdom adult population were 0.3 mg/kg bw per day at the mean and 0.9 mg/kg bw per day at the 97.5th percentile. For adults, the main contributors to the total anticipated exposure (>10%) were soft drinks (60%) and sauces, seasonings (e.g. curry powder, tandoori), pickles, relishes, chutney and piccalilli (18%).

The results of the EFSA tiered approach analyses are summarized in Table 3.

3.3.2 Food Standards Australia New Zealand

FSANZ included Sunset Yellow FCF in an overall survey of artificial colour use in foods in 2006. The foods and beverages examined were confectionery, ice cream, cheese, yoghurt, margarine, flavoured milk, flavoured soya beverages, soft drinks, cordials, fruit drinks, alcoholic drinks, biscuits, cakes, pastries, savoury snacks, breakfast cereals, pre-prepared meals, processed meats, sauces, toppings, jams/conserves and jelly. A small number of products that claimed to contain “no added colours” or “no artificial colour” were also sampled.

Assessments of dietary exposure to Sunset Yellow FCF were made for the Australian population aged 2 years and above, children aged 2–5 years, children aged 6–12 years, adolescents aged 13–18 years, adults aged 19–24 years and adults aged 25 years and above. The dietary exposures were estimated by combining usual patterns of food consumption, as derived from the 1995 National Nutrition

[1] The United Kingdom 97.5th percentile estimates herein are made from the 97.5th percentile estimate from beverages combined with the per capita estimates from all other coloured foods.

Table 3. EFSA dietary exposures to Sunset Yellow FCF

	Adults	Children 1.5–4.5 years old	Children 1–10 years old
Budget method	8.1		8.1[a]
Maximum permitted levels			
- Mean exposure	0.5	1.4	0.3–2.5
- Exposure at the 95th or 97.5th percentile	1.1	3.5	0.7–6.7
Maximum reported use levels			
- Mean exposure	0.3	1.1	0.2–2.1
- Exposure at the 95th or 97.5th percentile	0.9	3.2	0.6–5.8

[a] For children (age range not specified).

Survey, with analysed levels of the colour in foods. Estimates were made using two scenarios: the mean colours scenario and the maximum colours scenario.

In the mean colours scenario, mean analytical concentrations of Sunset Yellow FCF in survey foods were used. Both detected and "non-detect" results were used to derive the mean analytical concentrations. It was assumed that the use of mean food colour concentrations represents the most realistic exposure for consumers of a range of brands and varieties of particular foods over a period of time. In the maximum colours scenario, estimates were made by using the maximum analytical concentrations of Sunset Yellow FCF in the survey foods. The use of maximum food colour concentrations assumed that every processed food consumed contained the highest concentration of each colour detected in the survey, in this case, Sunset Yellow FCF. The report states that this model will significantly overestimate exposure to added colours, except where products containing food colours at the highest levels of use are consumed every day. The estimates made using the maximum colours scenario were not used by FSANZ in its overall evaluation of the safety of the use of artificial colours.

For the Australian population aged 2 years and older, the mean dietary exposure to Sunset Yellow FCF was 1.12 mg/day, with a 90th percentile exposure of 3.46 mg/day. The highest subpopulation mean was 1.82 mg/day, for 13- to 18-year-olds and 19- to 24-year-olds. The highest subpopulation 90th percentile exposure was 5.42 mg/day, for 19- to 24-year-olds. The highest estimates made using the maximum colours scenario were 7.57 mg/day at the mean and 23.43 mg/day at the 90th percentile, both for the 19- to 24-year-old subpopulation. The main contributors to dietary exposure were soft drinks, savoury snacks, ice cream and ice confections, and cordial.

These results are summarized in Table 4.

3.4 Conclusions

The estimates of dietary exposure to Sunset Yellow FCF calculated by EFSA were higher than those of FSANZ. The Committee concluded that this was

Table 4. FSANZ dietary exposures to Sunset Yellow FCF using the mean colours scenario

Population group	Mean		90th percentile	
	mg/person per day	mg/kg bw per day	mg/person per day	mg/kg bw per day
2–5 years old	0.81	0.05	1.97	0.12
6–12 years old	1.30	0.04	3.79	0.12
13–18 years old	1.82	0.03	5.19	0.09
19–24 years old	1.82	0.03	5.42	0.08
25+ years old	0.93	0.01	2.95	0.04
2+ years old	1.12	0.02	3.46	0.06

due to EFSA's use of maximum permitted and reported use levels in its tier 2 and tier 3 approaches, as opposed to FSANZ's use of the mean analysed levels for all foods. The latter approach is considered to be more realistic for preparing lifetime dietary exposure estimates. The Committee concluded that 6 mg/kg bw per day, the tier 3, 97.5th percentile EFSA estimate for children 1–10 years of age, should be considered for use in the safety assessment for Sunset Yellow FCF, as it represents the most conservative assessment. However, it recognized that the FSANZ estimate for children, 0.12 mg/kg bw per day, would be a more realistic dietary exposure estimate because of the extensive post-market analyses used in its preparation. The Committee concluded that the use of the realistic assessment was appropriate.

4. COMMENTS

4.1 Toxicological data

This summary of the available toxicological data combines the studies previously reviewed (Annex 1, references *8* and *59*) with recently published data.

Sunset Yellow FCF has a strongly anionic sulfonated moiety on the molecule, which limits its absorption from the gastrointestinal tract and results in excretion of greater than 95% of an orally administered dose in the faeces, with only about 3% absorbed as the parent compound. However, little of the ingested Sunset Yellow FCF present in faeces remains unchanged, with the extent of bacterial reduction of the azo group being dependent on the administered dose. Sunset Yellow FCF is metabolized by bacteria in the gastrointestinal tract to yield sulfanilic acid and 1-amino-2-naphthol-6-sulfonic acid, which are absorbed and metabolized to various *N*-acetylated forms.

Dietary administration of Sunset Yellow FCF to rats at doses up to 2330 mg/kg bw per day for 96 days was reported to cause diarrhoea and distension of the caecum at doses equal to and above 1500 mg/kg bw per day. Diarrhoea was

also observed in dogs after repeated oral exposure to Sunset Yellow FCF at a dose equivalent to 1250 mg/kg bw per day, but no details on the duration of the study or the sex of the animals were available. At 2330 mg/kg bw per day, increased relative weights of the testes were observed in rats, but without any accompanying histopathological lesions.

In contrast, a feeding study in rats completed in 2005 reported degenerative changes in the testes after 90 days of administration of Sunset Yellow FCF at doses equivalent to 250 or 1500 mg/kg bw per day. However, in that study, the purity of the administered Sunset Yellow FCF, which was purchased at a local market in India, was unknown. The presence of impurities in the administered material may explain the lack of concordance with the absence of any testicular lesions in studies of longer duration (80–104 weeks) and at higher doses (up to 2500 mg/kg bw per day) when using Sunset Yellow FCF of known purity. A consistent adverse finding in repeated-dose feeding studies in mice and rats was reduced body weight gain (>10%) at doses in excess of 2250 mg/kg bw per day in adult mice and in excess of 1500 mg/kg bw per day in adult rats. Reduced body weight gain was also observed in dogs after 2–3 months at a dose of 1250 mg/kg bw per day, but not in pigs after 98 days at dietary concentrations equivalent to a dose of 1000 mg/kg bw per day.

Eight long-term studies previously reviewed by the Committee showed no evidence of carcinogenicity at concentrations in the feed equivalent to an oral dose of up to 3000 mg/kg bw per day in mice and up to 2500 mg/kg bw per day in rats. The present review included five additional long-term repeated-dose studies that tested dietary concentrations of Sunset Yellow FCF equivalent to oral doses of 7500 mg/kg bw per day in mice and up to 2500 mg/kg bw per day in rats. The absence of carcinogenicity in the long-term bioassays is consistent with the weight of evidence from a range of in vitro and in vivo genotoxicity tests reviewed at this meeting and at previous meetings, indicating that Sunset Yellow FCF is not genotoxic.

No adverse effects on reproductive performance in mice and rats have been reported following dietary exposure to Sunset Yellow FCF at doses up to 1000 mg/kg bw per day. However, reduced rat pup survival was observed in comprehensive studies at doses of 1500 and 2500 mg/kg bw per day, with reduced pup body weight at doses of 750 mg/kg bw per day and above. Dam body weight was affected only at the highest tested dose of 2500 mg/kg bw per day. The NOAEL for reduced pup body weight was 375 mg/kg bw per day.

Teratogenicity studies in rats and rabbits at oral gavage doses up to 1000 mg/kg bw per day (highest tested dose) did not reveal any compound-related adverse effects.

There are reports suggesting that asthma or chronic idiopathic urticaria/angio-oedema in humans may be induced by oral exposure to Sunset Yellow FCF. However, most of these reports are characterized by poorly controlled challenge procedures. Although recent studies performed with better control conditions are available, no conclusion on idiosyncratic responses to Sunset Yellow FCF could be drawn from the available evidence.

The administration of six different food colours and a preservative, sodium benzoate, and the presence of multiple methodological deficiencies limited the value

of a recent study that investigated a possible relationship between hyperactivity in children and the consumption of beverages containing food colours. The use of mixtures in dosing studies does not permit any observed effects to be ascribed to individual components.

4.2 Assessment of dietary exposure

Estimates of dietary exposure to Sunset Yellow FCF prepared and published by EFSA and FSANZ were available to the Committee. The estimates of dietary exposure to Sunset Yellow FCF calculated by EFSA were much higher than those of FSANZ (0.12 mg/kg bw per day for children at the 90th percentile). The Committee concluded that this was due to the use of maximum reported use levels by EFSA, as opposed to the use of the mean analysed levels for all foods by FSANZ. The latter approach is considered to be more realistic for estimating lifetime dietary exposure. Because of the conservative assumptions used by EFSA in making the exposure estimates, the Committee concluded that the 97.5th percentile estimate of 6 mg/kg bw per day for children should be considered in the safety assessment for Sunset Yellow FCF in addition to the more realistic FSANZ estimate.

5. EVALUATION

The Committee noted that there were five additional long-term repeated-dose feeding studies that tested Sunset Yellow FCF at dietary concentrations equivalent to doses of 7500 mg/kg bw per day in mice and up to 2500 mg/kg bw per day in rats. One of these long-term studies in rats, which included in utero exposure, had a NOAEL of 375 mg/kg bw per day for reduced body weight among pups. On the basis of this NOAEL and the usual 100-fold uncertainty factor, the Committee established an ADI of 0–4 mg/kg bw (with rounding). The previous ADI of 0–2.5 mg/kg bw was withdrawn. The Committee noted that EFSA's conservative 97.5th percentile dietary exposure for children was above the ADI, whereas the 90th percentile dietary exposure for children, estimated by the more realistic FSANZ approach, was 3% of the upper limit of the ADI. In consequence, the Committee concluded that the dietary exposure of children to Sunset Yellow FCF does not present a health concern.

6. REFERENCES

Aboel-Zahab H et al. (1997). Physiological effects of some synthetic food colouring additives on rats. *Bollettino Chimico Farmaceutico*, 136:615–627.

Bateman B et al. (2004). The effects of a double blind, placebo controlled, artificial food colourings and benzoate preservative challenge on hyperactivity in a general population sample of preschool children. *Archives of Disease in Childhood*, 89:506–511.

Ching FP et al. (2005). Acute in-vivo histological effect of food colourants on some rat tissues. *Global Journal of Pure and Applied Sciences*, 11:241–247.

Chung KT, Fulk GE, Andrews AW (1981). Mutagenicity testing of some commonly used dyes. *Applied and Environmental Microbiology*, 42:641–648.

EC (1998). *Report on methodologies for the monitoring of food additive intake across the European Union. Final report submitted by the Task Coordinator, 16 January 1998.* Reports of a Working Group on Scientific Cooperation on Questions Relating to Food,

Task 4.2. Brussels, Belgium, European Commission, Directorate General III Industry (SCOOP/INT/REPORT/2).

EFSA (2009). Scientific opinion on the re-evaluation of Sunset Yellow FCF (E110) as a food additive. EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS). *The EFSA [European Food Safety Authority] Journal*, 7(1):1330 [44 pp.] (http://www.efsa.europa.eu/en/efsajournal/pub/1328.htm; accessed 29 May 2011).

EU (1994). European Parliament and Council Directive 94/36/EC of 30 June 1994 on colours for use in foodstuffs. *Official Journal of the European Communities*, L 237:13–29.

FAO/WHO (2009). Annex 2: Dose conversion table. In: *Principles and methods for the risk assessment of chemicals in food*. Geneva, Switzerland, Food and Agriculture Organization of the United Nations and World Health Organization (Environmental Health Criteria 240; http://whqlibdoc.who.int/ehc/WHO_EHC_240_14_eng_Annex2.pdf; accessed 29 May 2011).

FAO/WHO (2010). *Report of the Forty-second Session of the Codex Committee on Food Additives, Beijing, China, 15–19 March 2010*. Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (ALINORM 10/33/12; http://www.codexalimentarius.net/web/archives.jsp?year=10).

FSANZ (2008). *Survey of added colours in foods available in Australia. Study of concentrations in foods including dietary exposure assessment and risk characterisation*. Food Standards Australia New Zealand (http://www.foodstandards.gov.au/_srcfiles/Colours%20Survey_Final%20Report%2022%20Oct%2008%20_2_.pdf; accessed 29 May 2011).

Fuglsang G et al. (1994). Adverse reactions to food additives in children with atopic symptoms. *Allergy*, 49:31–37.

García-Ayllón M et al. (2006). Changes in liver and plasma acetylcholinesterase in rats with cirrhosis induced by bile duct ligation. *Hepatology*, 43:444–453.

Gibb C, Glover V, Sandler M (1987). In vitro inhibition of phenolsulphotransferase by food and drink constituents. *Biochemical Pharmacology*, 36:2325–2330.

Hannuksela M, Haahtela T (1987). Hypersensitivity reactions to food additives. *Allergy*, 42:561–575.

Haveland-Smith RB, Combes RD (1980). Screening of food dyes for genotoxic activity. *Food and Cosmetics Toxicology*, 18:285–290.

Helal EGE (2001). Progressive effects of the interaction of sodium nitrite and Sunset Yellow on different physiological parameters in albino rats. *Egyptian Journal of Hospital Medicine*, 2:23–46.

Helal EGE et al. (2000). Effect of some food colourants (synthetic and natural products) of young albino rats. I. Liver and kidney functions. *Egyptian Journal of Hospital Medicine*, 1:103–113.

Hess RA et al. (1988). Acute effects and long-term sequelae of 1,3-dinitrobenzene on male reproduction in the rat. II. Quantitative and qualitative histopathology of the testis. *Journal of Andrology*, 9:327–342.

Huff J (1982). FD&C Yellow No. 6: Condensation of the carcinogenesis bioassay technical report. *Environmental Health Perspectives*, 45:209–210.

Ibero M et al. (1982). Dyes, preservatives and salicylates in the induction of food intolerance and/or hypersensitivity in children. *Allergologia et Immunopathologia*, 10:263–268.

Ishidate M et al. (1984). Primary mutagenicity screening of food additives currently used in Japan. *Food and Chemical Toxicology*, 22:623–636.

Ivett JL et al. (1989). Chromosomal aberrations and sister chromatid exchange tests in Chinese hamster ovary cells in vitro. IV. Results with 15 chemicals. *Environmental and Molecular Mutagenesis*, 14:165–187.

Kluwe WM et al. (1984). Chronic kidney disease and organic chemical exposures: evaluations of causal relationships in humans and experimental animals. *Fundamentals of Applied Toxicology*, 4:889–901.

Kuno N, Mizutani T (2005). Influence of synthetic and natural food dyes on activities of CYP2A6, UGT1A6, and UGT2B7. *Journal of Toxicology and Environmental Health, Part A*, 68:1431–1444.

Mathur NRA et al. (2005a). Effect of Sunset Yellow on testis in rats. *Journal of Ecophysiology and Occupational Health*, 5:1–3.

Mathur NRA et al. (2005b). Sunset Yellow induced changes in the lipid profile in male albino rat. *Biochemical and Cellular Archives*, 5:197–200.

McCann D et al. (2007). Food additives and hyperactive behaviour in 3-year-old and 8/9-year-old children in the community: a randomized, double-blinded, placebo-controlled trial. *Lancet*, 370:1560–1567.

McGregor DB et al. (1988). Responses of the L5178Y tk+/tk– mouse lymphoma cell forward mutation assay: III. 72 coded chemicals. *Environmental and Molecular Mutagenesis*, 12:85–154 [erratum appears in *Environmental and Molecular Mutagenesis*, 12:345].

Mekkawy HAR et al. (2001). Effect of some food colours (natural and synthetic) on liver and kidney functions of male rats. *Toxicology Letters*, 123(Suppl. 1):37.

Mikkelsen H et al. (1978). Hypersensitivity reactions to food colours with special reference to the natural annatto extract (butter colour). *Archives of Toxicology: Supplement*, 1:141–143.

Muzzall JM, Cook WI (1979). Mutagenicity test of dyes used in cosmetics with the *Salmonella*/mammalian microsome test. *Mutation Research*, 67:1–8.

NCI/NTP (1982). *Carcinogenesis bioassay of FD&C Yellow No. 6 (CAS No. 2783-94-0).* Research Triangle Park, NC, and Bethesda, MD, USA, Department of Health and Human Services, National Cancer Institute, National Toxicology Program (National Toxicology Program Technical Report Series No. 208; http://ntp.niehs.nih.gov/ntp/htdocs/LT_rpts/tr208.pdf; accessed 29 May 2011).

Osman MY et al. (2002). Synthetic organic hard capsule food colouring agents: in vitro effect on human true and pseudo-cholinesterases. *British Journal of Biomedical Science*, 59:212–217.

Osman MY et al. (2004). Synthetic organic food colouring agents and their degraded products: effects on human and rat cholinesterases. *British Journal of Biomedical Science*, 61:128–132.

Pollock I, Warner JO (1990). Effect of artificial food colours on childhood behaviour. *Archives of Disease in Childhood*, 65:74–77.

Poul M et al. (2009). Lack of genotoxic effect of food dyes Amaranth, Sunset Yellow and Tartrazine and their metabolites in the gut micronucleus assay in mice. *Food and Chemical Toxicology*, 47:443–448.

Sasaki YF et al. (2002). The comet assay with 8 mouse organs: results with 39 currently used food additives. *Mutation Research*, 519:103–119.

Schultz-Ehrenburg U, Gilde O (1987). [Results of studies in chronic urticaria with special reference to nutritional factors.] *Zeitschrift für Hautkrankheiten*, 62:88–95.

Simon RA (2003). Adverse reactions to food additives. *Current Allergy and Asthma Reports*, 3:62–66.

Supramaniam G, Warner JO (1986). Artificial food additive intolerance in patients with angio-oedema and urticaria. *Lancet*, 2:907–909.

Tanaka T (1996). Reproductive and neurobehavioral effects of Sunset Yellow FCF administered to mice in the diet. *Toxicology and Industrial Health*, 12:69–79.

USFDA (1986). Mouse and rat studies undertaken by Bio/dynamics Labs and reported in *Federal Register*, 51(223):41766–41781. Provided to WHO by the United States Food and Drug Administration.

Watabe T et al. (1980). Reduction of sulphonated water-soluble azo dyes by micro-organisms from human faeces. *Food and Cosmetics Toxicology*, 18:349–352.

Westmoreland C, Gatehouse DG (1991).The differential clastogenicity of Solvent Yellow 14 and FD & C Yellow No. 6 in vivo in the rodent micronucleus test (observations on species and tissue specificity). *Carcinogenesis*, 12:1403–1408.

Wever J et al. (1989). Testing of Sunset Yellow and Orange II for genotoxicity in different laboratory animal species. *Environmental and Molecular Mutagenesis*, 13:271–276.

Wilson N, Scott A (1989). A double-blind assessment of additive intolerance in children using a 12 day challenge period at home. *Clinical and Experimental Allergy*, 19:267–272.

Worm M et al. (2000). Clinical relevance of food additives in adult patients with atopic dermatitis. *Clinical and Experimental Allergy*, 30:407–414.

Young E et al. (1987). The prevalence of reaction to food additives in a survey population. *Journal of the Royal College of Physicians*, 21(4):241–247.

CONTAMINANTS

CYANOGENIC GLYCOSIDES (addendum)

First draft prepared by

M. Feeley,[1] A. Agudo,[2] R. Bronson,[1] J. Edgar,[3] D. Grant,[4] T. Hambridge[5] and J. Schlatter[6]

[1] Food Directorate, Health Canada, Ottawa, Ontario, Canada
[2] Catalan Institute of Oncology, L'Hospitalet de Llobregat, Spain
[3] Honorary Fellow, CSIRO Food and Nutritional Sciences, North Ryde, Australia
[4] D.L. Grant & Associates Ltd, Ottawa, Ontario, Canada
[5] Food Standards Australia New Zealand, Canberra, Australia
[6] Nutritional and Toxicological Risks Section, Federal Office of Public Health, Zurich, Switzerland

1. EXPLANATION

Cyanogenic glycosides were evaluated by the Committee at its thirty-ninth meeting (Annex 1, reference *101*). The Committee noted that the potential toxicity of a food produced from a cyanogenic plant depends on the likelihood that its consumption will produce a concentration of hydrocyanic acid (HCN) that is toxic to exposed humans. Factors important to this potential toxicity include the following: 1) the plant may not be sufficiently detoxified during processing or preparation, and HCN may therefore remain in the food; 2) if the plant is consumed raw or is insufficiently processed, HCN may be released in the body, until the low pH of the stomach deactivates the plant β-glucosidase enzyme; and 3) a portion of the intact cyanogenic glycoside ingested with the food can be hydrolysed by β-glucosidase of the bacteria of the gut flora. The potential toxicity of HCN from ingested cyanogenic glycosides is dependent on a number of nutritional factors that are involved in detoxification mechanisms, including the availability of sulfur-containing amino acids and vitamin B12.

Although there were reports available showing associations between chronic exposure to cyanogenic glycosides and various diseases in humans (spastic paraparesis, tropical ataxic neuropathy and goitre), these associations were confounded by nutritional deficiencies. Therefore, the previous meeting of the Committee concluded that no causal relationship could be definitively established. Experimental animal studies were also assessed, and the Committee concluded that there were insufficient quantitative data on the level of exposure to cyanogenic glycosides or amount of HCN released on which to estimate a safe exposure level. The Committee also concluded that consumption of cassava flour containing total HCN at levels up to 10 mg/kg as specified in the current Codex standard for edible cassava flour (Codex Standard 176-1989) would not be associated with acute toxicity, but recommended that additional guidelines be developed for analytical methods for releasable HCN from cyanogenic glycoside–containing foods other than cassava.

The Third Session of the Codex Committee on Contaminants in Foods (CCCF) in 2009 requested that the Joint Food and Agriculture Organization of the United Nations (FAO)/World Health Organization (WHO) Expert Committee on Food Additives (JECFA) reconsider the available data on cyanogenic glycosides, advise on the public health implications of cyanogenic glycosides and their derivatives in food and decide whether risk assessment is feasible and appropriate (FAO/WHO, 2009a).

Table 1. Important cyanogenic glycoside–producing crops

Food	Major cyanogenic glycoside present	Cyanogen content (mg/kg as HCN)
Cassava (*Manihot esculenta*) – root	Linamarin	15–1000
Sorghum (*Sorghum vulgare*) – leaves	Dhurrin	750–790
Flax (*Linum usitatissimum*) – seed meal	Linamarin, linustatin, neolinustatin	360–390
Lima beans (*Phaseolus lunatus*)	—	2000–3000
Giant taro (*Alocasia macrorrhizos*) – leaves	Triglochinin	29–32
Bamboo (*Bambusa arundinacea*) – young shoots	Taxiphyllin	100–8000
Apple (*Malus* spp.) – seed	Amygdalin	690–790
Peach (*Prunus persica*) – kernel	Amygdalin	710–720
Apricot (*Prunus armeniaca*) – kernel	Amygdalin	785–813 89–2170 2.2 (juice)
Plum (*Prunus* spp.) – kernel	Amygdalin	696–764
Nectarine (*Prunus persica* var. *nucipersica*) – kernel	Amygdalin	196–209
Cherry (*Prunus* spp.)	Amygdalin	4.6 (juice)
Bitter almond (*Prunus dulcis*)	Amygdalin	4700

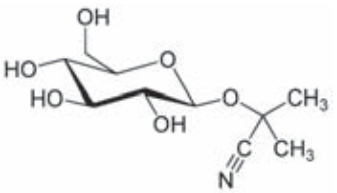
Source: NZFSA (2008)

1.1 Introduction

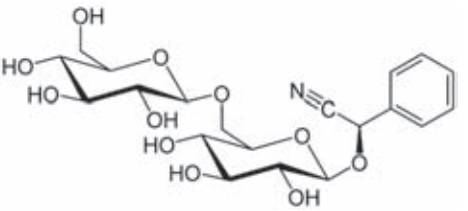
Cyanogenic glycosides, also referred to as “phytoanticipins” (Zagrobelny et al., 2004), are nitrogen-substituted secondary plant metabolites hypothesized to be part of an important evolutionary defence mechanism developed in a large number of plant species (Vetter, 2000; Bak et al., 2006). Another possible role for accumulated cyanogenic glycosides in certain angiosperm seeds is to provide a storage deposit of reduced nitrogen and sugar for the developing seedlings (Selmar, Grocholewski & Deigler, 1990). Up to 12 000 plant species are considered to be cyanogenic, or capable of producing up to 10 mg of HCN per kilogram of plant tissue (McMahon, White & Sayre, 1995; Sadasivam & Thayumanavan, 2003). More than 2600 species specifically produce cyanogenic glycosides, including up to 26 economically important crops (see Table 1). One example, cassava, has been described by FAO as the fourth most important crop for developing countries, with an estimated production in 2006 of 226 million tonnes; it is considered to be a major energy source for approximately 1 billion people living in the tropics.

A general structure of cyanogenic glycosides consists of a reactive α-hydroxynitrile component derived from one of six aliphatic and aromatic L-amino acid precursors (plus one non-protein amino acid cyclopentenyl-glycine), stabilized

Figure 1. Molecular structures of the cyanogenic glycosides linamarin and amygdalin

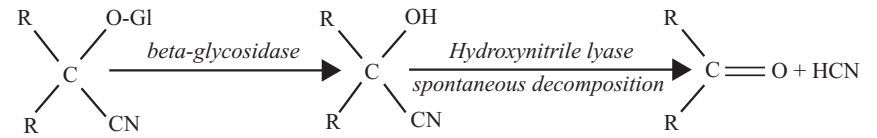


Linamarin



Amygdalin

Figure 2. General metabolic scheme for cyanogenic glycosides



Cyanogenic glycoside Cyanohydrin Ketone or aldehyde

Gl = glucose

through conjugation with either D-glucose or gentiobiose (6-*O*-β-D-glucopyranosyl-D-glucose) (β-glycosides of cyanohydrins) (see Figure 1 for examples) (Barceloux, 2009; Ganjewala et al., 2010; Jørgensen et al., 2011). Intact cyanogenic glycosides are specifically compartmentalized in plant tissues, separating them from their hydrolysing enzymes, β-glucosidases and hydroxynitrile lyases, with the cyanogenic glycosides in cell vacuoles and the enzymes in mesophyllic cells (Morant & Jørgensen, 2008; Sánchez-Pérez et al., 2009). Under conditions when plant cell structures are disrupted—for example, when plants are macerated by herbivores—β-glucosidases are released and come in contact with intact cyanogenic glycosides, resulting in the enzymatic cleavage of the carbohydrate moiety, D-glucose. The free α-hydroxynitrile can then either be enzymatically cleaved to a ketone or aldehyde and HCN by hydroxynitrile lyases or spontaneously dissociate, releasing HCN (Figure 2) (Conn, 1980; Barceloux, 2009). The aldehyde or ketone derived from α-hydroxynitrile cleavage depends on the specific cyanogenic glycoside, being benzaldehyde for amygdalin (L-mandelonitrile-β-D-glucuroniside-6-β-glucoside), prunasin (D,L-mandelonitrile-β-D-glucoside) and sambunigrin, *p*-hydroxybenzaldehyde for dhurrin and acetone for linamarin.

The cyanogenic glycosides and their breakdown products cyanohydrins and HCN are jointly known as cyanogens; the cyanogen content of a foodstuff is often expressed in terms of the HCN released by hydrolysis.

Following complete metabolism/hydrolysis, 1 g of linamarin (relative molecular mass = 247) could theoretically generate 109.3 mg HCN (equivalent to

105.2 mg cyanide). Similarly, 1 g of amygdalin would potentially yield 59.1 mg of HCN. Any potential toxicity of a cyanogenic glycoside would ultimately depend upon the concentration of HCN produced, as it is considered that mammalian tissues contain no significant amounts of β-glucosidase, and any ingested external β-glucosidase would be inactivated by the low pH of the stomach. However, two β-glucosidases with activity towards flavonoid glycosides have been isolated from human small intestine mucosa, lactase-phlorizin hydrolase and cytosolic β-glucosidase, indicating a role of these two enzymes from small intestine in flavonoid absorption and metabolism (Németh et al., 2003). Specifically, lactase-phlorizin hydrolase is anchored in the mucosal membrane in the brush border of the small intestine and has been shown to have broad substrate specificity for various glycosides. The other potential source of exposure to HCN from intact cyanogenic glycosides following ingestion would be by metabolism through bacterial flora of the gastrointestinal tract, which may also contain β-glucosidases (Kobawila et al., 2005). In addition, isolated rat small intestine uptake of D-glucose has been shown to be inhibited by prunasin, the major metabolite of amygdalin, suggesting competition for the glucose carrier site, sodium-dependent monosaccharide transporter (Wagner & Galey, 2003).

Of all cyanogenic plants, it has been suggested that clinically important human toxicity probably occurs only following the ingestion of cassava (Barceloux, 2009). The Codex Alimentarius Commission has developed and published standards for sweet and bitter cassava, edible cassava flour and gari (also spelt "garri", a product obtained from processing cassava tubers). The key aspects of these standards (http://www.codexalimentarius.net/web/standard_list.do?lang=en) are as follows:

- Sweet cassava is defined as a raw product containing less than 50 mg/kg of "hydrogen cyanide" (fresh weight basis) (Codex Standard 238-2003, AMD. 1-2005).
- Bitter varieties of cassava are those containing more than 50 mg/kg of cyanides expressed as hydrogen cyanide (fresh weight basis) (Codex Standard 300-2010).
- Edible cassava flour is defined as a product suitable for direct human consumption, and the level of "total hydrocyanic acid" in the flour must not exceed 10 mg/kg (Codex Standard 176-1989).
- For gari, another product for direct human consumption, the "total hydrocyanic acid" must not exceed 2 mg/kg as "free hydrocyanic acid" (Codex Standard 151-1989).

Neither the cassava flour nor gari standard specifies what variety of cassava (sweet or bitter) the products have to be derived from, only that both are prepared from the processing of cassava tubers (*Manihot esculenta* Crantz).

For bitter varieties of cassava, the Codex standard states that the following information must be made available to consumers at the point of sale or appear on a separate preparation statement:

- Cassava must not be eaten raw.
- Cassava shall be peeled, de-pithed, cut into pieces, rinsed and fully cooked before consumption.

- Cooking or rinsing water must not be consumed or used for other food preparation purposes.

At the Fifty-ninth Session of the Executive Committee of the Codex Alimentarius Commission (FAO/WHO, 2007), it was agreed that CCCF would consider the safety of the levels of HCN proposed in the standard for bitter cassava, with a view to a re-evaluation of cyanogenic glycosides by JECFA. CCCF at its Second Session (FAO/WHO, 2008a) considered the need for a re-evaluation of cyanogenic glycosides by JECFA and agreed that an electronic working group, led by Australia, would prepare a discussion paper, which should include an overview of available data on cyanogenic glycosides with a view to possible re-evaluation by JECFA. The *Discussion Paper on Cyanogenic Glycosides* (FAO/WHO, 2008b) was presented at the Third Session of CCCF in 2009 (FAO/WHO, 2009a) and recommended that JECFA reconsider the available data on cyanogenic glycosides and advise on the public health implications of cyanogenic glycosides and their derivatives in food—in particular, whether there are sufficient data to establish a health-based guidance value, such as an acute reference dose (ARfD) or tolerable daily intake (TDI), for cyanogenic glycosides or their derivatives present in food.

As it is considered that the toxicity of cyanogenic glycosides is directly related to the potential that consumption will produce a concentration of HCN (or cyanide, CN^-) that is toxic to humans, applicable toxicological data for cyanide were also reviewed separately.

1.2 Cyanohydrins

Cyanohydrins (α-hydroxynitriles) can occur in partially processed foods containing cyanogenic glycosides as an intermediary prior to the liberation of cyanide (see Figure 2 above). Aldehyde or ketone cyanohydrins can either spontaneously or enzymatically hydrolyse, resulting in the release of the cyanide moiety. For example, the cyanogenic potential of cassava flour is mainly due to the presence of linamarin, whereas cyanide in gari is present mainly as acetone cyanohydrin (Onabolu et al., 2002).

Acetone cyanohydrin (linamarin aglycone) decomposes spontaneously in the presence of water to acetone and HCN. In dilute aqueous solutions, acetone cyanohydrin will fully decompose. The half-life for decomposition is pH dependent and was calculated for a 0.1% solution as 57 minutes at pH 4.9, 28 minutes at pH 6.3 and 8 minutes at pH 6.8 (ICI, 1993). From the rate constant for decomposition at pH 7 and 26 °C of 4.47/hour, a half-life of 9 minutes was calculated (Ellington, Stancil & Payne, 1987).

Once absorbed, a dose of acetone cyanohydrin behaves in a manner identical to that of its molar equivalent in absorbed free cyanide. Cyanide concentrations in liver and brain of CD-1 mice were similar after a single intraperitoneal injection of an equimolar dose of acetone cyanohydrin or sodium cyanide. After a single injection of acetone cyanohydrin at 9 mg/kg body weight (bw), 108.0 ± 27.5 and 30.0 ± 4.6 mmol cyanide per kilogram were found in liver and brain, respectively. After a single injection of a 4.8 mg/kg bw dose of sodium cyanide, cyanide concentrations in

liver and brain were 87.8 ± 31.2 mmol cyanide per kilogram and 24.9 ± 4.8 mmol cyanide per kilogram, respectively (Willhite & Smith, 1981).

Lethal doses observed in experimental animals for acetone cyanohydrin approximate the molar equivalency of cyanide. The median lethal dose (LD_{50}) in mice for acetone cyanohydrin (equivalent to 2.65 mg of cyanide ion per kilogram) is similar to that of sodium cyanide, at 2.54 mg of cyanide ion per kilogram; mean time to death was 5 minutes for both compounds (Willhite & Smith, 1981).

Although cyanohydrin was not extensively reviewed as part of this cyanogenic glycoside monograph addendum, the overall conclusion of a recent review by the United States National Academies of Science was that acetone cyanohydrin behaves as its molar equivalent to cyanide both in vitro and in vivo (NAS, 2009). Therefore, it should be considered that any ingestion of cyanohydrin from foods containing partially degraded cyanogenic glycosides will liberate HCN based on a molar comparison.

2. BIOLOGICAL DATA: CYANOGENIC GLYCOSIDES

Searches for relevant publications were done utilizing various search engines and databases, including, but not limited to, RefWorks, PubMed, MedLine, SpringerLink.com, Wiley Interscience and Elsevier, for the time period 1990–2011. Methods involved keyword searches, journals, article titles, authors and previously cited references in related material.

2.1 Biochemical aspects

2.1.1 Absorption, distribution and excretion

Following oral administration, a proportion of ingested cyanogenic glycosides are absorbed and excreted intact in the urine (Barrett et al., 1977; Hernandez et al., 1995; Carlsson et al., 1999). The fraction unabsorbed can be enzymatically converted to HCN by microorganisms in the gastrointestinal tract (Frakes, Sharma & Willhite, 1986; Carlsson et al., 1999).

(a) Rats

Male albino Wistar rats (six per dose; approximately 100 g each) were administered an aqueous solution of linamarin at a dose of 10, 50, 100 or 350 mg/kg bw by stomach intubation. Urine samples were collected 24 hours later and analysed for cyanide and thiocyanate. A further three groups of rats each were injected (intravenously) with 10, 50 or 100 mg linamarin administered in 0.1 ml of 0.9% saline. Blood was drawn from the plexus orbitalis and assayed for linamarin (measured as bound HCN) over a 6-hour period after dosing. In the animals dosed orally, only approximately 2% of the linamarin dose was estimated to be excreted in the urine, based on analysis of both glycosidic and non-glycosidic cyanide. Dose-dependent increases in urinary excretion of both cyanide and thiocyanate were observed. In the animals dosed intravenously, intact linamarin was rapidly

eliminated from the blood with an estimated half-life of 90 minutes, with no significant difference between dose groups (Maduagwu, 1989).

(b) Hamsters

Female Golden Syrian hamsters (44–56 days old) were fasted overnight and dosed with either 0.44 mmol linamarin (n = 22) or amygdalin (n = 20) per kilogram body weight (108 and 201 mg/kg bw, respectively) by gavage (0.5 ml/100 g bw dosing volume). Blood samples were obtained by cardiac puncture 0.5, 1, 2, 3 and 4 hours after treatment and analysed for cyanide and thiocyanate. At least three hamsters were treated for each time point. Peak blood concentrations of cyanide were similar for both cyanogenic glycosides (116 and 130 nmol/ml, respectively, or 3.0 and 3.4 µg/ml) and were reached within 1–3 hours after dosing, with a subsequent rapid decline. At the last collection time point, blood thiocyanate levels were also similar (342 and 285 nmol/ml, respectively). All animals dosed with either cyanogenic glycoside exhibited symptoms of cyanide poisoning, with mortality rates of 18–20% (Frakes, Sharma & Willhite, 1986).

Additional in vitro studies by Frakes, Sharma & Willhite (1986) were conducted with a crude preparation of β-glucosidase isolated from hamster caecum to compare cyanide liberation from linamarin, amygdalin or prunasin at a concentration of 1 mmol/l. Under identical in vitro incubation conditions (40 minutes at 37 °C), approximately 4.5 times more cyanide was liberated when amygdalin was the substrate compared with linamarin, whereas prunasin was hydrolysed considerably more rapidly than amygdalin or linamarin. At the end of the incubation period, prunasin had yielded 708 nmol of cyanide, compared with only 294 nmol for amygdalin and 64 nmol for linamarin.

(c) Humans

Nine non-smoking adults (aged 25–59 years) consumed 1–4 kg of freshly boiled “sweet” cassava root over a 2-day period. On the 3rd day, urine samples were collected and analysed for linamarin and thiocyanate. The average urinary linamarin levels increased significantly from 2 to 68 µmol/l, whereas thiocyanate levels also increased from 12 to 22 µmol/l. In a second experiment, five non-smoking adults consumed an average of 480 g cassava root containing 21.2 mg cyanide equivalents per kilogram dry weight (approximately 71.2 mg/kg wet weight), and urine was collected at 12-hour intervals for up to 4 days for analysis of linamarin and thiocyanate. Peak urinary linamarin levels (19 µmol/l) were reached within 12 hours after cassava ingestion, returning to baseline levels by 48–60 hours. Minimal increases in urinary thiocyanate levels were observed (5 µmol/l before casava meal to 8 µmol/l after). It was estimated that approximately 28% of the ingested linamarin was excreted unmetabolized in the urine (Hernandez et al., 1995).

Twelve adult volunteers (average age 24 years) consumed 150 g of gari (dry granular cassava food) that had been presoaked for 5 minutes in cold water. The gari contained 24 mg extractable cyanide equivalents per kilogram dry weight as cyanohydrin, which could release up to 855 µmol of HCN. The linamarin content of the gari was described as negligible. Blood samples were collected 30 minutes

following the gari meal and then hourly for 8 hours. The mean plasma cyanide concentration at baseline was 6 µmol/l, inceasing to a peak of 12 µmol/l 8.6 hours following gari consumption and returning to baseline levels by 11.6 hours after dosing. The mean fraction of cyanide absorbed from the ingested gari was estimated at 13% (Oluwole, Onabolu & Sowunmi, 2002).

Fifteen healthy adult volunteers (non-smokers) consumed between 275 and 650 g of a cassava-based porridge (ugali), with urine and serum samples collected 24 and 48 hours after the meal for linamarin and thiocyanate analysis. A second group (n = 7) consumed a similar amount of ugali (230–750 g/person) and had urine samples collected five consecutive 24-hour periods after ingestion for linamarin analysis. Based on analysis of the cassava flour, the ingestion of cyanogenic glycosides (primarily linamarin) ranged from 243 to 574 µmol, or 4–10 µmol/kg bw (1–2.5 mg/kg bw per day). Approximately 21% of the ingested linamarin was excreted in the urine intact during the first 24 hours after ingestion. All subjects exhibited a mean increase in serum thiocyanate of 44 µmol/l (range 24–74 µmol/l). In the second group, an average of 23% of the ingested linamarin was excreted unmetabolized in the urine, mainly within the first 24 hours after ingestion (15% by 8 hours, 7% within the next 16 hours). The authors concluded that as no correlation was found between linamarin exposure and increases in serum thiocyanate levels in group 1 and excess thiocyanate excretion in group 2, individual differences in linamarin to cyanide conversion are more important for the exposure than the amount ingested (Carlsson et al., 1999).

Additional description of these studies is provided in section 2.3.

Linamarin was detected in all urine samples (n = 59) collected from school-age children from Mozambique who had consumed underprocessed cassava root twice a day. The mean concentration was 211 µmol/l (approximately 52 mg/l) (Brimer & Rosling, 1993).

2.1.2 Biotransformation

The biotransformation of cyanogenic glycosides involves two main steps: first, cleavage of the carbohydrate moiety by β-glucosidases and then subsequent enzymatic or spontaneous dissociation of the cyanohydrin to the corresponding aldehyde or ketone and HCN (see Figure 2 above). Only partial absorption of intact cyanogenic glycosides takes place from the gastrointestinal tract, as demonstrated from various studies in experimental animals and humans (Sakata et al., 1987; Brimer & Rosling, 1993; Hernandez et al., 1995). Thiocyanate excretion in the urine suggests that up to 10–20% of the linamarin can be enzymatically converted in the gastrointestinal tract to release cyanide.

In isolated perfused segments of small intestine of male Sprague-Dawley rat, approximately 10% of a 25 mg/l concentration of amygdalin was converted to prunasin. No appreciable formation of prunasin was detected in sections of ileum exposed to amygdalin under identical experimental conditions. In addition, incubation of a suspension of rat caecal contents with amygdalin resulted in formation of both prunasin and benzaldehyde, the latter suggesting further metabolism of prunasin to mandelonitrile. A reduction in formation of prunasin in small intestine isolated

from rats that had received prior antibiotic treatment suggested that metabolism of amygdalin to prunasin occurs in the proximal part of the small intestine, followed by hydrolysis of amygdalin to glucose, benzaldehyde and cyanide by the microflora of the large intestine (Strugala, Rauws & Elbers, 1986).

Crude suspensions of human faecal specimens (approximately 3 g) were prepared from 100 non-smoking Korean adults and incubated with amygdalin at a concentration of 0.5 mmol/l for 1 hour at 37 °C. At the end of the reaction time, the suspension was extracted with ethyl acetate and analysed by thin-layer chromatography (TLC). It was reported that, on average, the conversion of amygdalin to benzaldehyde occurred at a rate of 2.4 mmol/hour per gram and was proportional to the conversion of other herbal components that require metabolism by β-D-glucosidases (Kim et al., 2008).

As part of a United States National Cancer Institute clinical trial, amygdalin was administered by intravenous injection to six patients undergoing treatment for cancer at a dose of 4.5 g/m^2 per day (approximately 120 mg/kg bw per day) for 21 days. High peak plasma levels of amygdalin (up to 1160 µg/ml) were observed, with elimination half-lives ranging from 44 to 157 minutes. Recoveries of amygdalin in 24-hour urine collections conducted during the 1st week of therapy showed average daily excretion values ranging between 62% and 96% of the administered dose. The cyanide concentration in blood was described as “essentially undetectable”. For an additional six patients who were given 0.5 g amygdalin orally 3 times daily for a period of 7 days, urinary levels of amygdalin were between 8% and 32% of the ingested dose (Moertel et al., 1981).

2.2 Toxicological studies

The toxicological studies on cyanogenic glycosides are summarized in Table 2.

2.2.1 Acute toxicity

Acute toxicity and mortality induced by various cyanogenic glycosides in experimental animals are directly related to, and influenced by, factors associated with the release and detoxification of HCN.

The oral LD_{50} of amygdalin in Wistar rats was reported to be 880 mg/kg bw. However, when amygdalin at 600 mg/kg bw was administered orally with 10 units of β-glucosidase, all the rats died (Adewusi & Oke, 1985).

Female Fischer rats (150–200 g) were given a single oral dose of amygdalin (<400–1100 mg/kg bw) and observed for up to 14 days. The LD_{50} of amygdalin was calculated to be approximately 522 mg/kg bw, with the first sign of neurological damage appearing 80 minutes after administration (Newton et al., 1981).

Conventional Sprague-Dawley strain rats (450 and 550 g) experienced lethargy and convulsions, and usually died within 2–5 hours, following a 600 mg/kg bw single oral dose of amygdalin. Blood cyanide concentrations were significantly elevated (2.6–4.5 µg/ml) in all rats displaying signs of toxicity. In contrast, germ-free rats of the same strain showed no adverse effects after the same dose of

Table 2. Summary of toxicological studies on cyanogenic glycosides

Chemical	Dose	Effect	Species	Reference
Linamarin, amygdalin	0.44 mmol, single, gavage	Toxicity; 18–20% mortality	Hamster	Frakes et al. (1986)
Amygdalin	Gavage, single, variable	LD_{50} 880 mg/kg bw	Rat	Adewuse & Oke (1985)
Linamarin	Gavage, single, variable	LD_{50} 450 mg/kg bw	Rat	Oke (1979)
Amygdalin	Gavage, single, <400–1100 mg/kg bw	LD_{50} 522 mg/kg bw	Rat	Newton et al. (1981)
Linamarin	Gavage, single, 250, 500 mg/kg bw	Acute toxicity, lethality	Rat	Philbrick, Hill & Alexander (1981)
Prunasin	Gavage, single, 300–1000 mg/kg bw	LD_{50} 560 mg/kg bw	Rat	Sakata et al. (1987)
Linamarin	Gavage, continual, 94 mg/kg bw per day, 5 weeks	Blood lactic acid increase	Rat	Philbrick, Hill & Alexander (1977)
Amygdalin	Gavage, 20 mg/kg bw per day, 14 days	Increased blood cyanide, histopathology of liver	Rat	Oyewole & Olayinka (2009)
Fresh cassava	Feed 50% or 70% cassava = 0.07 or 0.102 mg/day as cyanide; 1 year	Decreased body weight, decreased serum insulin, histopathology of pancreas and liver, decreased motor coordination	Rat	Mathangi, Mohan & Namasivayam (1999); Mathangi et al. (2000)
Amygdalin	Single, gavage, 200–275 mg/kg bw, GD 8	Material toxicity, fetal abnormalities	Hamster	Willhite (1982)
Linamarin	Single, gavage, 70–140 mg/kg bw, GD 8	Fetal defects	Hamster	Frakes, Sharma & Willhite (1985)
Cassava meal (+ low-protein diet)	Diet, GDs 3–15, equivalent to 1.6 + 21 mg/kg bw per day as cyanide	Decreased fetal ossification (21 mg/kg bw per day), decreased pup body weight	Hamster	Frakes et al. (1986)

GD, gestation day

amygdalin, and blood cyanide levels did not differ from those of control animals (<0.4 µg/ml) (Carter, McLafferty & Goldman, 1980).

In vitro studies demonstrated that when amygdalin was incubated with 1 g of human faeces, 53% of the available HCN was released by hydrolysis, whereas incubation of amygdalin in human gastric juice samples at pH values below 4.1 resulted in no HCN release. Urinary excretion of thiocyanate was substantially reduced in rats pretreated with neomycin 3 days prior to amygdalin exposure (Newton et al., 1981).

Previously, it was reported by the Committee that the oral LD_{50} of linamarin in rats was 450 mg/kg bw (Annex 1, reference *102*).

Male Wistar rats (average body weight, 91 g) were fed a diet supplemented with methionine (0.3% of diet) for 2 weeks, at which time some animals received the same diet without the methionine for 4 days. Animals then received linamarin given as a single oral dose (250 or 500 mg/kg bw). At the higher linamarin dose, all animals died within 5 hours after dosing. Biochemical and physiological changes observed in these rats included severe metabolic acidosis, decreased cytochrome oxidase activities, atrial fibrillation and decreased respiratory rates (Philbrick, Hill & Alexander, 1981).

Twelve male Wistar rats (average body weight, 91 g) received linamarin at 500 mg/kg bw, and 10 rats received cyanide (administered as potassium cyanide) at 6 mg/kg bw, by gavage. When the animals became sufficiently docile after dosing, electrocardiograph and respiratory traces were recorded. Ataxia, apnoea, docility, paresis and severe cyanosis were observed in all animals within 4 minutes after the oral dose of potassium cyanide. Similar symptoms were observed in rats dosed with linamarin within 120 minutes after dosing. All dosed animals eventually died. The authors concluded that similarity of electrocardiograph and respiratory tracings from linamarin- and cyanide-dosed rats suggests that cyanide, released during digestion or following absorption of linamarin, was a major factor in the toxicity of the cyanogenic glycoside (Philbrick, Hill & Alexander, 1977).

Male Wistar rats (8 weeks old; 180–250 g; six per dose group) were given oral doses of prunasin (95% purity) at 300–1000 mg/kg bw and monitored for 24 hours. Mortality was observed in animals dosed with prunasin at 500, 630 and 1000 mg/kg bw, with animals dying within 5 hours after dosing. The LD_{50} was estimated to be 560 mg/kg bw. In animals dosed with prunasin at 300–500 mg/kg bw, between 30% and 40% of the dose was recovered intact in the urine within 24 hours after dosing (Sakata et al., 1987).

2.2.2 Short-term studies of toxicity

(a) Mice

Albino mice (15–35 g; 13 per group) were maintained on diets containing either 70% corn or 70% gari for 5 weeks. Animals from each group were removed every subsequent week of exposure, and liver mitochondria were collected for analysis. The HCN content of the diets was not provided, although the authors

stated that the cyanide content of the gari flour was typically 10.6–22.1 mg/kg. After 3 weeks, there was a slight reduction of mitochondrial respiratory control ratio in the animals on the gari diet compared with the animals on the corn diet; however, mitochondrial oxygen consumption rates were similar between groups. The respiratory control ratio is considered to be an indicator of the degree of coupling (or uncoupling) of oxidative phosphorylation. Between weeks 3 and 4, the specific activity of cytochrome c oxidase was decreased by approximately 30% in the gari-fed mice, but by week 5, similar activity was seen in both dose groups (Ezeji et al., 2009).

(b) Rats

Groups of male albino Sprague-Dawley rats (100–200 g; six per group) were fed diets containing 71% boiled cassava as a carbohydrate source ad libitum for 60 days. An additional group received the same diet plus were treated with 50% ethanol at 4 g/kg bw per day (by gavage) over the same time period. At termination, blood and liver samples were collected for clinical and histopathological analysis (liver). The cyanide content of the cooked cassava was estimated to be 7–9 mg/kg. Hepatic rhodanese activity was significantly increased in both cassava diet groups, which was reflected in the significant increases in serum thiocyanate concentrations (0.92 mg/dl controls, 2.46 mg/dl cassava, 2.06 mg/dl cassava plus ethanol). Blood cyanide concentration was also increased in both the cassava and cassava plus alcohol dose groups (57% and 28%, respectively). It was reported by the authors that co-administration of cassava and alcohol reduced the alcohol-induced degeneration of hepatocytes and presence of fatty vacuoles (Boby & Indira, 2004).

In a similar study, the cyanogenic glycoside–rich fraction was extracted from cassava root and given by gavage to a group of male albino rats (100–120 g; $n = 6$) daily for 60 days at 180 mg/kg bw per day. One kilogram of fresh cassava yielded 10 g of the cyanogenic glycoside–rich fraction, with the 180 mg/kg bw dose reported to be equivalent to 71 g of cooked cassava. Additional groups of rats (six per dose group) were also treated with 50% ethanol (4 g/kg bw per day) by gavage, alone or in combination with the cyanogenic glycoside fraction. Indices of hepatic lipid peroxidation (malondialdehyde, hydroperoxides, conjugated dienes) were significantly increased in all dose groups compared with controls, with the highest increase observed in the combined dose group (e.g. 128% increase in hepatic malondialdehyde with ethanol, 109% increase with cyanogenic glycoside fraction, 195% increase in combined dose group). Hepatic glutathione content was decreased in a manner similar to lipid peroxidation. In comparison, rats that had consumed a diet supplemented with 71% cooked cassava showed less lipid peroxidation effects of the ethanol treatment. The authors attributed this to the presence of antioxidants in the cooked cassava (vitamin C and A) and/or decreased alcohol absorption due to cassava root fibre (Boby & Indira, 2003).

Twenty male weanling Wistar rats (average body weight, 36 g) were divided equally into four groups and fed semipurified diets ad libitum for 5 weeks. An additional two groups of rats received the same diet without methionine supplementation (0.3%). All rats were dosed with linamarin at 94 mg/kg bw per

day by gavage, which was estimated to be equivalent to the minimum lethal dose of cyanide for rats when administered intravenously. At sacrifice, urine and blood samples were collected from each rat and analysed for thiocyanate (urine), pyruvate and lactate (blood). No mortality or signs of toxicity were observed, whereas urinary thiocyanate excretion was significantly increased in all linamarin-dosed animals. Animals maintained on the methionine-deficient diet had reduced feed intake efficiency, which was not affected by linamarin. Whole blood lactic acid levels were also significantly increased in the rats dosed with linamarin, with minimal influence of dietary methionine (Philbrick, Hill & Alexander, 1977).

Weanling male Wistar albino rats (six per group) were provided either caloric equivalent maize or cassava-based diets supplemented with either adequate (16.5%) or deficient (8.0%) protein for 4 weeks, after which all the rats (at age 8 weeks) were changed to the control diet for another 4 weeks. Additional rats were provided maize-based diets with or without adequate protein and supplemented with a sublethal concentration (750 mg/kg) of potassium cyanide. Protein-replete diets were additionally supplemented with methionine (0.2%). An intraperitoneal glucose tolerance test was performed when the rats were aged 4, 8 and 12 weeks, whereas plasma glucose and thiocyanate levels were also assessed. Thiocyanate levels increased (from 0.068 to 0.184 mmol/l) in both cassava diet groups, with no effect of protein deficiency. Glucose clearance, as assessed from the incremental area under the 2-hour glucose concentration versus time curve, was decreased approximately 50% in the animals on the cassava plus adequate protein diet compared with controls. In animals maintained on the cassava plus inadequate protein diet, a 250% decrease in glucose clearance was observed compared with control diet animals. Following 4 weeks on control diets, both previous cassava-fed groups still showed decreased glucose clearance compared with controls (approximately 50% decrease). In the rats fed diets with added potassium cyanide, similar plasma thiocyanate increases were noted (from 0.060 to 0.150 mmol/l), whereas the rats fed a protein-sufficient diet supplemented with methionine and potassium cyanide showed no glucose tolerance difference compared with controls. The authors concluded that continuous cassava intake and protein malnutrition have independent and additive effects on the development of glucose intolerance, which are not consistently reversible (Akanji & Famuyiwa, 1993).

Male Sprague-Dawley rats (10–14 weeks of age) weighing between 134 and 142 g (six per group) were fed for 10 weeks with either standard rat chow or diets composed of toasted cassava granules produced from cassava mash fermented for varied periods (0–72 hours). Fermenting cassava progressively reduces both the total cyanogen and crude protein content of the cassava granules. After the 10-week feeding period, the rats were euthanized, gross necropsy lesions were noted, and the liver, kidney, pancreas and brain tissues were processed for histopathology. All rats fed exclusively with the cassava-based diet exhibited various clinical signs of toxicity by the 3rd week, including loss of appetite, generalized weakness, wasting of the hindlimb muscles, posterior paresis and reduced sensitivity to noise. More severe effects were seen in the rats fed the diet produced from the 24-hour fermented cassava mash, even when compared with rats fed unfermented cassava. Similar body weight declines were also noted in all rats fed the cassava diets. All dose groups with cassava exhibited mortality during the course of the 10-week

feeding (33–50%), with all rats in the 24-hour fermentation group dying before the end of the 10 weeks. Histopathological abnormalities were also noted in various organs of the cassava-fed groups, consisting mainly of cellular vacuolization, degeneration and necrosis. More severe lesions were reported in the cerebrum, cerebellum and kidney for the 24-hour fermentation diet group compared with the other cassava groups (combination of lowest total cyanogen and protein content). The noted clinical observations and toxicological effects were described by the authors as being consistent with a combination of protein deficiency and chronic cyanide toxicity (Ihedioha & Chineme, 2003).

Male albino Wistar strain rats (150–165 g; seven per dose group) were administered amygdalin at a dose of 20 mg/kg bw daily (by oral gavage) for 14 days, whereas additional amygdalin-treated animals (same dose) were co-administered oral doses of hydroxocobalamin at 25 or 50 mg/kg bw. At termination, blood and liver samples were collected for analysis. One animal in the amygdalin-only dose group died before the end of the study period, whereas all animals in the combined dose groups survived to termination. Blood cyanide levels were significantly reduced in the combined treatment groups compared with the amygdalin-only dose group (15.23 µmol/l versus 5.10 and 4.55 µmol/l, respectively). Histological alterations observed in the amygdalin-only dose group (parenchyma necrosis, marked portal inflammation) were not observed in the combined treatment groups (Oyewole & Olayinka, 2009).

Male albino Wistar rats (190–250 g) were fed for 1–21 days with a control diet, control diet supplemented with either fresh cassava root or cassava root that had been processed into flour, or only fresh cassava root and cassava flour. No HCN concentrations of the various diets were reported. Serum thiocyanate concentrations were significantly increased in the rats fed fresh cassava for 21 days compared with control diet animals (0.9 mg/dl versus 0.4 mg/dl); however, no significant change in thyroid gland weight or evidence of hypotrophy or hyperplasia of the thyroid glands was observed. In rats fed a standard diet with added processed cassava, no changes in serum thiocyanate levels were seen (Kittivachra, 2006).

Male Wistar rats (3 months old; 25 per diet group) were maintained for 15 days on either a standard diet or diets in which cyanide-free cassava flour represented 50% by weight of the diet. Additional cassava-based diets were prepared with added 0.19% potassium cyanide and/or DL-methionine (0.3%) (final diet concentration). All prepared diets were reported to be isoenergetic. After 15 days, approximately half the rats in each diet group (n = 10–12) were rendered diabetic through intraperitoneal injection of five doses of streptozotocin (20 mg/kg bw). At 3 and 7 days after the last streptozotocin injection, blood samples were obtained from half the rats per diet group following an overnight fast and analysed for glucose, insulin and erythrocyte antioxidant enzyme activities (Yessoufou et al., 2006). Induction of diabetes increased blood glucose levels in all rats, including those on control diets, with the highest levels seen in rats that had been fed diets containing cyanide-free cassava flour with or without potassium cyanide. Addition of methionine to the diet did not prevent hyperglycaemia but did reduce the serum glucose levels in rats fed cassava diets plus potassium cyanide to the increase seen in rats fed control diets with induced diabetes. Induction of diabetes also decreased serum insulin in all rats, regardless of the diet group, with the greatest decreases

observed in rats fed the cyanide-free cassava flour with or without potassium cyanide. As with serum glucose, supplementing the latter diets with methionine caused an insulin decrease similar to that observed in rats fed the control diet. All erythrocyte antioxidant enzyme activities (superoxide dismutase, glutathione peroxidase, glutathione reductase) were increased in rats fed diets containing cyanide-free cassava flour as compared with control diet animals. In addition, the decreased activity seen in these enzymes in control diet animals rendered diabetic was largely absent in the rats fed the cassava. The authors considered that feeding a cassava-containing diet with cyanide and no supplemental methionine appeared to aggravate diabetes.

(c) Dogs

Three groups of male dogs (n = 6; subspecies not identified) were fed a control diet with rice as the carbohydrate source, a diet with cassava (gari) as the carbohydrate source (10 mg HCN per kilogram cooked food) or a control rice-based diet to which sufficient sodium cyanide was added at feeding time to release 10.8 mg HCN per kilogram cooked food for 14 weeks. For both dose groups, the daily HCN dose was reported by IPCS (2004) as 1.08 mg/kg bw (100 g diet consumed per day). Urine and plasma samples were collected throughout the feeding period and at study termination, as well as various tissue samples for histopathology. Plasma thiocyanate concentrations were increased to a similar extent in the gari and sodium cyanide diets, except at termination, when the animals fed the sodium cyanide diet showed the highest levels (48.6 versus 119 mmol/ml, respectively). Urine thiocyanate levels were consistently higher in the sodium cyanide–dosed dogs compared with the gari diet group. Protein loss in the urine was observed in both experimental dose groups, but with greater amounts in the gari-fed dogs compared with the sodium cyanide dose group (766 µmol/ml versus 483 µmol/ml, respectively, at week 14). Histopathological changes seen in the kidney consisted of congestion, vacuolization, swelling and rupture of the epithelial cells of the proximal tubules in the dogs fed the gari diet, whereas in the dogs fed the cyanide diet, the epithelial cells of the proximal tubules showed no swelling or vacuolization. The only histopathological changes in the liver were found in the dogs fed the gari diet and consisted of congestion and periportal vacuolation. Dogs fed the cassava diet also exhibited haemorrhage, pyknotic nuclei and swelling of muscle fibres in the myocardium, whereas dogs fed the sodium cyanide diet did not show any cardiovascular effects (Kamala, 1993).

2.2.3 Long-term studies of toxicity and carcinogenicity

Weanling inbred Wistar strain albino rats (both sexes) were randomly divided into three groups of 10 animals each and maintained on diets of normal rat chow (diet I), 50% fresh cassava and 50% normal rat feed (diet II) or 75% fresh cassava and 25% normal rat feed (diet III). The cassava contained, on average, a cyanide concentration of 10 mg/kg, which resulted in exposures of 0.075 or 0.102 mg of cyanide per animal per day in diets II and III, respectively. Animals were maintained on their diets for 1 year, at which time the animals were sacrificed and tissues collected for histopathology. By 3 months and until study termination, body weights were significantly decreased by approximately 25% in both cassava diet groups

compared with controls. No significant differences were noted with respect to blood glucose or serum amylase levels, whereas serum insulin levels were decreased approximately 50% in only the diet III animals and only at 6 months. Mild atrophy of the acini with minimal focal dilatation of ducts was noted in the pancreas of diet III animals; however, there were no changes consistent with acute or chronic pancreatitis. Histopathology of the liver showed similar features of toxic hepatitis with hyperplasia and microvascular changes in the hepatocytes in both cassava diet groups. After 5 months, motor coordination, as assessed by the rotor rod test, was significantly decreased in both cassava diet groups compared with controls (Mathangi, Mohan & Namasivayam, 1999; Mathangi et al., 2000).

2.2.4 Genotoxicity

No information was available to the Committee on the genotoxicity of cyanogenic glycosides.

2.2.5 Reproductive and developmental toxicity

(a) Multigeneration studies

No information was available.

(b) Developmental toxicity

Hamsters

Female Golden Syrian hamsters (strain LVG) were treated by gavage with a single dose of D,L-amygdalin (200–275 mg/kg bw) on gestation day (GD) 8. Animals were sacrificed on GD 14 and fetuses examined for developmental malformations. Signs of maternal toxicity (hyperpnoea, dyspnoea, ataxia and tremors) were noted in animals dosed with amygdalin at and above 250 mg/kg bw. An increased incidence of fetal abnormalities (mainly encephalocele and rib fusions or bifurcations) was noted with increased amygdalin dosing, ranging from a 4% incidence of abnormal fetuses at 200 mg/kg bw up to 32% at a dose of 275 mg/kg bw. A teratogenic dose of amygdalin (275 mg/kg bw) delivered by intraperitoneal injection or co-exposure to oral doses of amygdalin plus intraperitoneal doses of sodium thiosulfate caused no developmental effects (Willhite, 1982).

In the same experiment as described above (Willhite, 1982), eight pregnant hamsters were treated on GD 8 with a single oral dose of D-prunasin (177 mg/kg bw). The author reported that similar types of abnormalities as with amygdalin were observed in 15% of the fetuses examined. Prunasin is the mono-aglycone of amygdalin.

Female Golden Syrian hamsters (8–12 per dose group), beginning on GD 3, were fed low-protein diets (4.1%) with or without added cassava meal obtained from high- or low-cyanide cassava varieties. Animals were terminated on GD 15 and fetuses examined for malformations. The total cyanide concentration was, on average, 7.9 mmol/kg in the high-cyanide cassava diet and 0.6 mmol/kg in the low-cyanide cassava diet (corresponding to 205 and 15.6 mg/kg diet, respectively),

with most of the cyanide present as cyanogenic glycoside. Estimated daily cyanide equivalent intake by the dams was 1.6 and 21 mg/kg bw per day for the low- and high-cyanide cassava diets, respectively. At study termination, maternal blood from both the low- and high-cyanide cassava diets had elevated levels of thiocyanate compared with controls (66.4 and 95.5 nmol/ml, respectively), with similar concentrations observed in whole fetal homogenates (84.2 and 69.8 nmol/g, respectively). Urinary thiocyanate levels were significantly different between the groups, with the high-cyanide cassava diet group excreting 49 µmol/day compared with 3.6 µmol/day for the low-cyanide cassava group. No significant differences were noted in numbers of implantations, resorptions or live fetuses per litter. Delayed fetal ossification, unrelated to the low protein content of the diet, was significantly increased in the groups fed low- or high-cyanide cassava compared with controls. The percentage of pups per litter with a body weight 25% below average weight of littermates (runting) was also increased in the cassava diet groups (2–3% controls, 6% low-cyanide diet, 12% high-cyanide diet) (Frakes et al., 1986).

The Committee previously evaluated a study in which pregnant Golden hamsters (Lak:LVG(SYR)) (n = 10–13) were dosed by gavage on GD 8 with linamarin (>95% purity) at 0, 70, 100, 120 or 140 mg/kg bw. On GD 15, the animals were sacrificed and fetuses removed for internal and external examination. There was a dose-dependent increase in maternal animals exhibiting signs of intoxication (dyspnoea, ataxia, tremors, hypothermia) beginning at 100 mg/kg bw, with mortality of 1 of 11 and 2 of 13 animals in the 120 and 140 mg/kg bw dose groups, respectively, within 2 hours after dosing. Signs of maternal toxicity were largely absent within 3 hours after dosing, whereas maternal weight gain was decreased by only approximately 20% in the 120 mg/kg bw dose group (Frakes, Sharma & Willhite, 1985). In a related study previously reviewed by the Committee, subcutaneous infusion by osmotic minipump of sodium cyanide at 78–81 mg/kg bw per day between GD 6 and GD 9 also produced signs of toxicity in pregnant Golden hamsters (weight loss, ataxia, dyspnoea), but significant incidences of malformations observed in the offspring were not found to be correlated to any symptom of maternal toxicity (Doherty, Ferm & Smith, 1982). In the Frakes, Sharma & Willhite (1985) study, litters with prenatal deaths, number of live fetuses per litter and fetal body weight were not significantly different in any linamarin dose group compared with controls. The incidence of gross internal fetal defects was not significantly different in any of the test groups compared with controls; however, there was a dose-dependent increase in skeletal defects (missing presacral vertebrae, agenesis of one or both 13th ribs) noted in fetuses from linamarin-treated dams (Frakes, Sharma & Willhite, 1985). Additional details of the dose–response assessment for this study are provided in section 10.

2.2.6 Special studies

(a) Immunological and neurological effects

(i) In vitro

Rat PC12 (neural phaeochromocytoma) cells were incubated in different concentrations of linamarin (0.01–5.00 mmol/l) or potassium cyanide (0.001–0.250

mmol/l) for 48 hours and tested for evidence of cytotoxicity. Approximately equal toxicities were observed at a linamarin concentration of 2 mmol/l and at a potassium cyanide concentration of 0.05 mmol/l. Co-incubation of PC12 cells with cytochalasin B, a glucose transport inhibitor, at a concentration of 0.1 mmol/l reduced linamarin-induced cytotoxicity by approximately 50% (30% versus 15% cytotoxicity at a linamarin concentration of 0.5 mmol/l, respectively) (Sreeja et al., 2003).

(ii) In vivo

Male albino Wistar strain rats (approximately 2 months old) were divided into three groups of 10 animals and provided a control diet, a 25% control diet plus 75% fresh cassava root (8–10 mg cyanide equivalents per kilogram) or a protein-deficient diet (75% potato by weight) for 30 days. Open-field behaviour was tested in the animals every week, and, after the last testing on day 31, the hypothalamus was removed for analysis of neurotransmitters. Behavioural patterns in the open field showed that the cassava-fed animals did not acclimatize to the open-field environment, even after repeated exposures, whereas cassava consumption also decreased the basal levels of hypothalamic catecholamines (dopamine, norepinephrine and serotonin). Somewhat similar, but not identical, behavioural changes were also noted in the animals fed the protein-deficient diet; however, the decreases in catecholamines seen with cassava were not observed (Mathangi & Namasivayam, 2000b).

Female Long-Evans rats (35–42 days old; $n = 12$) were exposed to acetone cyanohydrin in drinking-water at a concentration of 10 mmol/l for 14 days, followed by acetone cyanohydrin in drinking-water at a concentration of 20 mmol/l for an additional 42 days. For the last 21 days, the rats were also deprived of standard food and instead given cassava starch (no indication provided as to HCN equivalents). Following total food deprivation for 24 hours, osmotic pumps were implanted in the animals, delivering 0 ($n = 6$) or 50 ($n = 6$) µmol of acetone cyanohydrin per kilogram body weight per hour for 24 hours. Animals were returned to their normal diet and had behavioural assessments done 1 and 6 days later. Rats exposed only to acetone cyanohydrin in drinking-water plus the cassava starch diet for 3 weeks did not show any overt neurological deficits (open-field behaviour). Subsequent exposure of these animals to 50 µmol acetone cyanohydrin per kilogram body weight per hour for 24 hours by osmotic pump caused behavioural effects indicative of acute toxicity, including immobility, decreased response to stimuli and abnormal walking postures. Recovery of the treated animals occurred after the 24-hour exposure period, and no consistent motor effects could be demonstrated by quantitative assessment 6 days after exposure. The authors concluded that the results do not support the hypothesis that acetone cyanohydrin is the causal agent for konzo, a neurotoxic disease in humans associated with consumption of underprocessed cassava (Soler-Martín et al., 2010).

Male heterozygous nude rats (Crl:NIH-Fox1 mu/Fox 1+), 5–7 weeks old, were assigned ($n = 5$) to isonitrogenous diets containing all amino acids or a diet lacking 75% of the sulfur-containing amino acid content relative to the control diet. Rats of each diet group were also treated intraperitoneally with linamarin at 50–200 mg/kg bw per day (given as graded doses: 50 mg/kg bw for 4 days, 100 mg/kg bw for

1 day and 200 mg/kg bw for 6 days) or sodium cyanate at 200 mg/kg bw per day for 2 weeks. Physical signs of intoxication associated with linamarin were described as minor and consisted of hindlimb tremors mostly observed in animals under sulfur-containing amino acid–deficient diet near the end of the treatment period. Sodium cyanate–treated animals lost approximately 20% of their original body weight after 1 week of treatment and presented with more severe signs of toxicity, consisting of both hindlimb weakness and gait abnormalities, regardless of the type of diet, after 2 days of treatment. Spinal cord proteomic assessment suggested that proteomic changes induced by linamarin under a sulfur-containing amino acid deficiency diet condition were qualitatively similar to those in animals treated with sodium cyanate on the normal amino acid diet. However, ultrastructural analysis of the cervical spinal cord and proximal segments of sciatic nerves by electron microscopy did not indicate significant treatment-related effects. The authors concluded that additional human studies are needed to explore whether serum levels of linamarin as well as those of cyanate and carbamoylated proteins correlate with increased risk for konzo among cassava-reliant populations (Kassa et al., 2011).

2.3 Observations in humans

2.3.1 Biomarkers of exposure

The levels of some biomarkers, mainly thiocyanate concentrations in urine and blood, have been reported in many papers reviewed below dealing with acute poisoning and neurological disorders associated with cyanide and cyanogenic glycosides. In this section, we include only studies that assessed the relationship between dietary exposure to cyanide or cyanogens due to cassava consumption and the level of biomarkers, both in cross-sectional studies in the population and in experimental studies in volunteers.

No studies were found that quantitatively linked measured levels of biomarkers to numerical estimates of dietary exposure. The information evaluated indicated that consumers of cassava have higher urinary thiocyanate levels compared with individuals who never consume cassava; weekly consumers have levels between those of daily consumers and non-consumers (Mlingi et al., 1998; Chiwona-Karltun et al., 2000; Cardoso et al., 2004). Frequent (e.g. twice per day) or high levels of consumption of cassava can result in low levels of urinary thiocyanate (<100 µmol/l) when effective processing reduces the levels of cyanogenic glycosides (Mlingi et al., 1996). Even those with frequent or high consumption of cassava or consumers of bitter cassava can have low levels of urinary thiocyanate if the cassava is processed effectively (Peterson et al., 1995; Banea-Mayambu et al., 2000). Consumption of varieties of cassava with low levels of HCN results in lower levels of urinary thiocyanate and linamarin (Mlingi et al., 1998). Levels of urinary inorganic sulfate are higher for those who consume a broader range of foods in the diet (other than cassava), which include other amino acids (including sulfur-containing amino acids). Biomarker levels tend to be seasonal, with peaks occurring during times of cassava harvest when cassava consumption is higher. Occupational exposure for people working in cassava processing plants results in higher levels of urinary and serum thiocyanate compared with frequent consumers of cassava (Okafor, Okorowkwo & Maduagwu, 2002).

(a) Studies in volunteers: metabolic fate of cyanogenic glycosides

One experimental study was conducted in 1993 to elucidate the characteristics of toxic exposure from cyanogenic glycosides in cassava consumed in Cuba. In the first experiment, nine non-smoking healthy adults who had not eaten cassava in the preceding week provided a urine specimen on day 1. They then received peeled root pieces of sweet cassava to be consumed boiled in water as a staple food for the two subsequent dinners and lunches (amounts consumed 1–4 kg per 48 hours). A sample of this cassava was taken for analysis. A second urine sample was collected on day 3. For comparison, seven smoking adults (15–20 cigarettes/day) provided urine specimens as well. Linamarin was absent in the urine of smokers and present in minimal amounts in the urine of non-smokers before consumption of cassava, but urinary concentrations were substantially increased after consumption (mean from 2 to 68 µmol/l). Urinary thiocyanate levels were much higher in smokers (119 µmol/l) than among non-smokers; among the latter, thiocyanate levels increased only slightly after cassava consumption (mean from 12 to 22 µmol/l). Urinary inorganic sulfate levels were higher among non-smokers, but with similar levels before and after cassava consumption (10.7 and 9.5 mmol/l). In a second experiment, five non-smokers (laboratory staff) made nine consecutive 12-hour urine collections. In the first hour of the third period, all ate 500 g fresh weight boiled cassava (same origin as in previous experiment). The cyanogenic glycoside content of cassava was as follows: glycosides, 19.8 mg HCN equivalents per kilogram dry weight (535 µmol glycosides per kilogram); cyanohydrins, 0.2 mg HCN equivalents per kilogram dry weight; and HCN, 1.2 mg/kg dry weight (32 µmol HCN per kilogram). As the dry weight of consumed cassava was 143 g, they ingested a mean of 76.5 µmol of cyanogenic glycosides and 4.6 µmol of HCN. The mean total urinary excretion of linamarin was 28%, with a peak in the first 12-hour sample. The urinary thiocyanate level was 5 µmol/l before consumption, increased modestly to 8 µmol/l in period 8 and returned to 4 µmol/l at the end (period 9). The main finding of this study was that consumption of boiled fresh roots of sweet cassava resulted in a dietary exposure to cyanogenic glycosides that did not cause a corresponding exposure to dietary cyanide. Almost one third of the glycoside consumed was recovered in the urine; the remaining two thirds may have been excreted in the urine as a metabolite without having released cyanide or was excreted unabsorbed in the faeces. The authors emphasized that the form of cyanogenic substance occurring in the cassava products is as important for the resulting cyanide exposure as the total amount of cyanogens. The intermediate breakdown product of cyanogenic glycosides, cyanohydrins, has been implicated as the main source of cyanide exposure from poorly processed bitter cassava, but was almost absent in the boiled cassava consumed in this study. The levels of cyanogenic glycosides in the boiled cassava were slightly above the safe limit of 10 mg HCN equivalents per kilogram dry weight, but this intake resulted in a negligibly increased cyanide exposure (Hernández et al., 1995).

The metabolic fate of linamarin in cassava was investigated in two groups of volunteers in the United Republic of Tanzania. The first group consisted of 15 healthy non-smoking laboratory staff members who had not consumed any cassava product during the preceding 5 days. Cassava flour was obtained at the village markets; it was mixed with boiled water and immediately served with vegetable

relish and meat (ugali). The consumed portions of cassava ranged from 275 to 650 g (80–189 g dry weight). Two consecutive 24-hour urine samples were collected after the meal, as well as blood samples before and 48 hours after the meal. In the flour samples used to make the meal, the mean content was 82 mg HCN equivalents per kilogram dry weight for cyanogenic glycosides, 0.7 mg HCN equivalents per kilogram dry weight for cyanohydrins and 1 mg/kg dry weight for HCN (respectively 2216, 19 and 27 µmol HCN equivalents per kilogram dry weight). The amount of total cyanogenic glycosides consumed ranged from 243 to 574 µmol (4–10 µmol/kg bw). The mean excretion of linamarin was 22% (21% during the first 24-hour period), and the increase in serum thiocyanate level was 44 µmol/l (from 34 to 78 µmol/l after 48 hours). Linamarin excretion correlated with cassava flour intake but not with the serum thiocyanate level. A second group of seven healthy non-smoking laboratory staff members who had not consumed cassava during the preceding 5 days collected urine 24 hours before a cassava meal and for five consecutive 24-hour periods following the meal; the first 24-hour period was further divided into two fractions of 8 and 16 hours. The mean amounts of cyanogenic glycosides from the consumed cassava flour were 80 mg HCN equivalents per kilogram dry weight for cyanogenic glycosides, 0.9 mg HCN equivalents per kilogram dry weight for cyanohydrins and 1.2 mg/kg dry weight for HCN (or 2162, 24 and 32 µmol HCN equivalents per kilogram dry weight, respectively). The subjects consumed 230–750 g cassava ugali, corresponding to 69–225 g dry weight, giving an intake of 203–669 µmol linamarin (4–9 µmol/kg bw). The mean proportion of linamarin excreted in the 48-hour period was 28% (15% in the first 8 hours and 23% during the first 24 hours). Six out of seven subjects had a clear increase in thiocyanate excretion that peaked around day 3 and returned to almost initial values on day 5. It must be remembered that thiocyanate serves only as a semiquantitative estimate of cyanide exposure, as about 20% of a cyanide dose is converted to other metabolites. The authors concluded that there is substantial absorption of linamarin, assumed to take place in the gut, which can lead to dietary cyanide exposure. The urinary excretion of linamarin was very rapid, whereas the increase in thiocyanate excretion was delayed. The absence of a correlation between linamarin exposure and serum thiocyanate level in the first group and excess thiocyanate excretion in the second group suggests that individual differences in linamarin to cyanide conversion are more important for the exposure than the amount ingested. About one quarter of the cyanogenic potential in the form of linamarin in a given cassava product will give rise to cyanide exposure in the consumer (Carlsson et al., 1999).

(b) Studies in the general population

In a study carried out on 217 women from western United Republic of Tanzania to assess the relationship between cassava and goitre (Mlingi et al., 1996; reviewed in the section on “goitre and thyroid function” below), the consumption of cassava and methods of processing were also analysed in relation to the cyanogen content of cassava flour and urinary excretion of thiocyanate. Fresh cassava roots were soaked in water for 3 days and sun dried for 3 more days and then were either milled or pounded to obtain flour. The amounts of cyanogens in flour according to the processing method (milled versus pounded) were as follows: glycosides, 35.8 versus 37.0 mg HCN equivalents per kilogram dry weight; cyanohydrins, 8.2 versus

11.2 mg HCN equivalents per kilogram dry weight; HCN, 7.9 versus 7.3 mg/kg dry weight; and total cyanogens, 49.9 versus 53.9 mg HCN equivalents per kilogram dry weight. The difference was statistically significant only for cyanohydrins. The mean urinary thiocyanate level was 128 µmol/l (median 70 µg/dl); overall, 38% of women had a urinary thiocyanate level of 100 µmol/l or above (assumed to be an indicator of high exposure to cyanide). This proportion was not associated with the frequency of cassava consumption, but was significantly lower among women who often consumed cassava flour obtained by milling (26%) compared with those who did so only sometimes or never (74%). It is assumed that cyanide exposure from sufficiently processed cassava results from the remaining amount of glycosides and the intermediate breakdown product, cyanohydrin. The latter compound can spontaneously decompose to volatile HCN 1) if the moisture content falls below 12–13% and if pH rises above 6 or 2) if the flour is stored for a long time. The processing experiment revealed higher amounts of cyanohydrins in the flour obtained by pounding compared with the milled one. This is important, as cyanohydrins are believed to quantitatively yield cyanide when broken down in the alkaline environment of the gut (Tylleskär et al., 1992). Mlingi et al. (1996) suggested that the inverse relationship between urinary thiocyanate levels and frequency of milling can be explained, because mechanical milling may enhance the breakdown of the remaining cyanohydrins and evaporate the formed HCN by heating and airing of the flour. Moreover, as milling requires dry cassava root pieces in order for the mill not to clog, only roots sufficiently dried are accepted by the milling machine, whereas traditional pounding allows production of flour from relatively wet roots.

A study was carried out to ascertain the amount of cyanide exposure from consumption of cassava products in the United Republic of Tanzania. A food frequency questionnaire was applied to 206 schoolchildren (mean age 14 years) from six suburbs of Dar es Salaam, 193 of whom also provided a urine sample. Seventy cassava flour samples were also obtained from local markets, with an average concentration of 9.4 mg HCN equivalents per kilogram dry weight of total cyanogens and a range from 0 to 79 mg HCN equivalents per kilogram dry weight (mean values of 6.4 mg cyanogenic glycosides, 2.02 mg cyanohydrins and 3.21 mg HCN per kilogram dry weight). Almost all subjects (97%) had eaten cassava during the previous week; the mean number of cassava meals was 4.8 per week. The mean urinary thiocyanate level was 36 µmol/l, and the mean urinary linamarin level was 18 µmol/l. Multiple regression analysis revealed a statistically significant association only between consumption of boiled cassava pieces and urinary thiocyanate levels (each meal increased the level by 6.7 µmol/l), whereas linamarin levels in urine were associated only with consumption of fried cassava pieces (increased level of 1.5 µmol/l per meal). The urinary thiocyanate level in this study was very low compared with the mean level found in populations consuming insufficiently processed bitter cassava roots in southern United Republic of Tanzania and was in the same range as the level found in Swedish schoolchildren. The roots consumed were from non-bitter cassava varieties with low levels of cyanogenic glycosides. In spite of direct sun drying being considered an ineffective method of removing cyanogens in cassava, the mean total cyanogen level in makopa samples (sun-dried cassava root pieces) was lower than 10 mg HCN equivalents per kilogram dry weight (Mlingi et al., 1998).

In 1996, in a study in a district of northern Malawi, information on usual diet and urine specimens were collected for 175 women with a mean age of 40 years. Fifty-five per cent of the women reported daily consumption of cassava porridge with fish, legumes or pulses (kondowole) during the previous week. The mean urinary concentrations were 14 µmol/l for linamarin and 50 µmol/l for thiocyanate. The three women who admitted having experienced acute effects from shortcut cassava processing had urinary thiocyanate levels of 60, 118 and 410 µmol/l, respectively. The low urinary linamarin levels found indicate that almost all linamarin had been broken down or leached out of the roots during soaking. The mean urinary thiocyanate level is about a tenth of the levels observed in consumers of insufficiently processed bitter cassava roots and in the same range as the levels in non-smoking Swedish subjects. The relatively frequent consumption of fish probably provided the studied population with somewhat better protein status compared with other cassava-eating populations (Chiwona-Karltun et al., 2000).

One study was carried out in three villages of mid-western Nigeria, identified as a major cassava-producing area, to investigate whether high cassava production indicates high consumption and high dietary cyanide exposure. Forty-two households were selected; samples of ready-to-eat cassava products were gathered in 36 of them, and food questionnaires and urine specimens were obtained from 110 women and children above 2 years of age in these households. Cassava food products were eaten at least once a day in 88% of households, with an average of 14 servings in the preceding week. Mean energy intake was about 9.2 MJ for adults and 6.7 MJ for children (13% provided by cassava in average); the average daily intake of proteins was 24 g for adults and 19 g for children. Mean urinary excretions were as follows: 51 µmol/l for thiocyanate, 20 µmol/l for linamarin and 7 mmol/l for inorganic sulfate. The total amount of cyanogens in food samples ranged from 0 to 62 mg HCN equivalents per kilogram dry weight; 10 out of 51 samples had cyanogen levels above 10 mg HCN equivalents per kilogram. The urinary thiocyanate and linamarin levels found are compatible with modest intakes of cyanogens from cassava and much lower than the levels observed in cassava-consuming populations where epidemics of konzo have been reported (Onabolu et al., 2001a).

Another study considered the biochemical changes and toxicological implications that may be associated with large-scale cassava processing and ingestion in Ogbomoso Metropolis, Oyo State, Nigeria. The study was carried out among 20 subjects: 10 cassava processors (those who have been involved in cassava processing for more than 6 months), 5 frequent consumers of cassava foods and 5 infrequent consumers of cassava foods; all of them were female non-smokers aged 24–50 years and without overt signs of sickness or disease. Urine and blood samples were taken to assess serum and urinary thiocyanate and blood cyanide concentrations. The mean urinary thiocyanate level of the processors (153.5 µmol/l) was 2.2 and 2.6 times higher than those of frequent (70.1 µmol/l) and infrequent (59.3 µmol/l) cassava food consumers. A similar trend in the serum thiocyanate level was also observed; the mean concentrations were 126.7 µmol/l for processors, 68.4 µmol/l for frequent consumers and 54.7 µmol/l for infrequent consumers. The mean blood cyanide level was also higher in the processors (7.7 µmol/l) than in the frequent (3.1 µmol/l) and infrequent (2.3 µmol/l)

consumers. These results suggest that occupational and dietary exposures of humans to cyanide with concomitant biochemical and toxicological effects do occur in connection with large-scale cassava processing and ingestion of cassava-based food products. This occupational exposure to cyanide could be mainly from water intake, inhalation and skin absorption of HCN discharged into the environment in the course of processing (Okafor, Okorowkwo & Maduagwu, 2002).

One study carried out in 2001 combined the information from flour cyanide and urinary thiocyanate concentrations to calculate cassava flour intake and aimed to compare results from sites where new cases of konzo had recently occurred with control sites where konzo had not been reported recently. Four sites in the provinces of Nampula and Zambezia in Mozambique were selected, two of them with recent konzo cases and two with old previous konzo cases. The investigators administered a questionnaire and collected 30 cassava flour samples from houses in each site. The questionnaire asked about food consumed in the previous 24 hours. The investigators also obtained 30 samples of urine from schoolchildren in the same sites on the same day, together with information on the proportion of schoolchildren who had consumed cassava on the previous day. Beyond the comparison between the two areas concerning konzo occurrence, the main purpose of the study was to provide a calculation method to derive the amount of daily consumption of cassava flour from a given urinary excretion of thiocyanate. The urinary thiocyanate level was assumed to be measured in the population, and other data can be either directly measured or taken from previous studies. The following data were needed: cyanide content of cassava flour (mg HCN per kilogram); proportion of HCN converted to thiocyanate (27%, from previous studies); baseline thiocyanate urinary excretion in non-smoking non-cassava eaters (25 µmol/l, from previous studies); and daily urine volume (600 ml in this study). The mean urinary thiocyanate concentrations were 301 and 355 µmol/l in the two sites with recent konzo and ranged from 47 to 85 µmol/l in the old konzo sites. The mean cyanide concentration in flour was 18–28 mg/kg in recent konzo sites and 26–41 mg/kg in old konzo sites. The maximum cassava flour intake was estimated to be 720–920 g/day in recent konzo sites and 23–140 g/day in control sites. According to the authors, calculation of the maximum daily cassava intake allows one to distinguish between populations who are almost totally reliant on cassava and who are therefore at risk of contracting konzo and those who have a broader, safer diet. In contrast, they suggested that measurements of total cyanide in cassava flour alone do not necessarily give a good idea of cyanide consumption (Cardoso et al., 2004).

A general summary has been provided for biomarkers measured in various populations exposed to cyanogenic glycosides through the diet (Table 3).

2.3.2 Health effects

Acute toxicity results when the absorption rate of HCN exceeds its metabolic detoxification. Acute toxic effects result primarily from inhibition of cellular respiration and consequent histotoxic anoxia, which can eventually lead to death. Several disorders observed in cassava-eating populations have been attributed to chronic exposure to dietary cyanide. These include mainly neurological disorders such as spastic paraparesis and tropical ataxic neuropathy, but also diabetes and

Table 3. Summary of biomarkers of exposure to cyanogenic glycosides and related key population and dietary features

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (µmol/l)	Serum thiocyanate Mean ± SD or range (µmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (µmol/l)
Central African Republic[1]	NS	Adults and children	13	Konzo affected	Cassava was the staple food; 100% consumed cassava in previous 24 h; shortcuts taken in processing, taking out fermentation	189 ± 48	—	—	—	—
Central African Republic[2]	NS	Schoolchildren	48	Western village; 50% had goitre	Insufficiently processed cassava; 100% consumed cassava in previous 24 h	239 ± 19	—	—	—	—

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (µmol/l)	Serum thiocyanate Mean ± SD or range (µmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (µmol/l)
		Population	84	Western village; 50% had goitre	Insufficiently processed cassava; 100% consumed cassava in previous 24 h	231 ± 17	—	—	—	—
		Schoolchildren	15	Central village; 28% had goitre	Sufficiently processed cassava; 100% consumed cassava in previous 24 h	48 ± 8	—	—	—	—
		Population	15	Central village; 28% had goitre	Sufficiently processed cassava; 93% consumed cassava in previous 24 h	81 ± 9	—	—	—	—

Table 3 (contd)

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (μmol/l)	Serum thiocyanate Mean ± SD or range (μmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (μmol/l)
		Schoolchildren	61	City; 22% had goitre	NS	65 ± 6	—	—	—	—
Congo[3]	1986	Children 7–14 years	46	NS	Bitter cassava adequately processed; % consuming cassava in previous 24 h: 87% morning, 76% midday, 94% evening	50 ± 7	—	—	5.0 ± 0.6	—
	1988		31	NS	Bitter cassava not properly processed; % consuming cassava in previous 24 h: 90% morning, 44% midday, 95% evening	757 ± 85	—	—	5.8 ± 0.9	—

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (µmol/l)	Serum thiocyanate Mean ± SD or range (µmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (µmol/l)
Cuba[4]	NS	Adults	7	Smokers (15–20 cigarettes/day)	NS	119 ± 17	—	—	5.7 ± 0.6	0 ± 0
			9	Non-smokers	Before cassava consumption	12 ± 2	—	—	10.7 ± 0.8	2 ± 1
			9	Non-smokers	After cassava consumption	22 ± 2	—	—	9.5 ± 1.8	68 ± 16
Malawi[5]	1996	Adult women (mean 40 years; range 15–70 years)	176	NS	81% reported consuming staple cassava meal daily during the previous week; 51% twice daily; 38% consumed maize daily due to rains destroying much of the cassava crop	50 ± 4 Range 2–410; *n* = 23–100 By frequency of cassava consumption: Daily 57 ± 7 Weekly 48 ± 6 Never 29 ± 5	—	—	—	14 ± 1 Range 3–39 By frequency of cassava consumption: Daily 14 ± 1 Weekly 13 ± 1 Never 13 ± 1

Table 3 (contd)

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (µmol/l)	Serum thiocyanate Mean ± SD or range (µmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (µmol/l)
Mozambique[6]	2001	Schoolchildren	30	Recent konzo: site 1	Maximum 920 g cassava flour consumed per day[c] at 18 mg/kg cyanide; 84% consuming cassava in previous 24 h	301	—	—	—	—
			30	Recent konzo: site 2	Maximum 720 g cassava flour consumed per day[c] at 27 mg/kg cyanide; 93% consuming cassava in previous 24 h	351	—	—	—	—

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (µmol/l)	Serum thiocyanate Mean ± SD or range (µmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (µmol/l)
			30	No recent konzo: site 1	Maximum 58 g cassava flour consumed per day[c] at 39 mg/kg cyanide; 42% consuming cassava in previous 24 h	72	—	—	—	—
			30	No recent konzo: site 2	Maximum 140 g cassava flour consumed per day[c] at 26 mg/kg cyanide; 60% consuming cassava in previous 24 h	85	—	—	—	—

Table 3 (contd)

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (μmol/l)	Serum thiocyanate Mean ± SD or range (μmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (μmol/l)
Mozambique[7]	1982	Schoolchildren (mean age 8.1 years)	30	Previous spastic paraparesis	Diet reliant on bitter cassava after drought, 1–2 weeks of sun drying	125 ± 15 mmol/mol	—	1.17 ± 0.05	0.41 ± 0.04 mol/mol creatinine	—
Mozambique	1983	Schoolchildren (mean age 9.0 years)	31	Previous spastic paraparesis; live in village	Diet reliant on bitter cassava after drought, 1 month of sun drying	83.9 ± 9.3 mmol/mol	—	1.42 ± 0.09	0.73 ± 0.08	—
Mozambique	1983	Schoolchildren (mean age 9.3 years)	30	No spastic paraparesis; live in administrative centre	High cassava eaters	52.0 ± 12.6 mmol/mol	—	2.42 ± 0.36	1.34 ± 0.17	—
Mozambique	1983	Schoolchildren (mean age 7.1 years)	28	No spastic paraparesis; live in city	Consumed virtually no cassava	8.9 ± 2.7 mmol/mol	—	1.97 ± 0.12	1.36 ± 0.10	—

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (µmol/l)	Serum thiocyanate Mean ± SD or range (µmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (µmol/l)
Sweden (controls)	1982	Schoolchildren (mean age 8.6 years)	17	NS	Non-cassava consuming	3.4 ± 0.6 mmol/mol	—	2.56 ± 0.16	2.16 ± 0.14 mol/mol creatinine	—
Mozambique[8]	1981	Patients with spastic paraparesis	246 (specimens)	NS	Drought 2 years, insufficiently processed bitter cassava consumed	—	324 ± 18	—	—	—
		Controls in village of patients	22	NS	Drought 2 years, insufficiently processed bitter cassava consumed	—	288 ± 23	—	—	—
		Pregnant women	14	NS	Non-cassava consumers	—	15	—	—	—
Glasgow		Smokers	NS	Normal range	—	—	70–150	—	—	—
		Non-smokers	NS	Normal range	—	—	10–40	—	—	—
Sweden		Smokers	NS	Normal range	—	—	7–237	—	—	—

Table 3 (contd)

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (μmol/l)	Serum thiocyanate Mean ± SD or range (μmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (μmol/l)
		Non-smokers	NS	Normal range	—	—	8–77	—	—	—
Mozambique[9]	1993	Children	77	Communities with konzo following war	Insufficiently processed bitter cassava	207 ± 14	—	—	4877 ± 392 μmol/l	80 ± 9
Mozambique[10]	2006	NS	12	Drought; people with konzo	NS	479 ± 70	—	—	—	—
	2005		12			528 ± 137	—	—	—	—
	2006		10			380 ± 98	—	—	—	—
	2005		27			272 ± 33	—	—	—	—
	2006		25			289 ± 27	—	—	—	—
	2005		20			379 ± 71	—	—	—	—
	2006		30			321 ± 23	—	—	—	—
Mozambique[11]	1997	Children 6–11 years	29	Study at end of cassava harvest	Cassava flour (from bitter cassava) eaten had mean total cyanogens concentration of 67 ± 39 mg/kg	512 ± 277	—	—	—	—

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (µmol/l)	Serum thiocyanate Mean ± SD or range (µmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (µmol/l)
Mozambique[12]	1999	Schoolchildren	150	5 areas surveyed; 8–17% children had ankle clonus	Consumed cassava flour containing mean total cyanogens 26–186 mg/kg	362 ± 346 339 ± 358 225 ± 190 384 ± 230 298 ± 168	—	—	—	—
Nigeria[13]	NS	Adults (females 24–50 years)	10	Cassava processing plant workers	Cassava consumption not specified	153.50 ± 25.21	126.73 ± 12.4	—	—	—
			5	Non-cassava processing plant workers	Frequent cassava consumers (at least once per day)	70.1 ± 21.8	68.4 ± 18.3	—	—	—
			5	Non-cassava processing plant workers	Occasional cassava consumers	59.3 ± 17.4	54.7 ± 13.2	—	—	—

Table 3 (contd)

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (μmol/l)	Serum thiocyanate Mean ± SD or range (μmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (μmol/l)
Nigeria[14]	NS	Women and children above 2 years of age	110 did FFQ, 118 weighed food records	During period of high cassava production	88% of households consume cassava daily from the FFQ; yam the staple food; mean energy contribution of cassava is 13% (from weighed food records); total cyanogens from all foods consumed ranged from 0 to 62 mg HCN equivalents/kg dry weight	51 ± 35	—	—	7 ± 4	20 ± 11

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (µmol/l)	Serum thiocyanate Mean ± SD or range (µmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (µmol/l)
United Republic of Tanzania[15]	1993	Adults	15	NS	Consumed 275–650 g stiff porridge (ugali) (mean ± SD in mg cyanide equivalents/kg dry weight: cyanogenic glycosides 82 ± 1, cyanohydrins 0.7 ± 0.1, HCN 1.0 ± 0.2); dietary exposure to cyanogenic glycosides 243–574 µmol or 4–10 µmol/kg bw	—	At 0 h (after eating porridge): 34 ± 23; at 48 h: 78 ± 28	—	—	At 0 h: 0 ± 0 µmol/24 h; at 24 h: 85 ± 62 µmol/24 h; at 48 h: 3 ± 5 µmol/24 h

Table 3 (contd)

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (μmol/l)	Serum thiocyanate Mean ± SD or range (μmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (μmol/l)
			7		Consumed 230–750 g stiff porridge (ugali) (mean ± SD in mg cyanide equivalents/ kg dry weight: cyanogenic glycosides 80 ± 2, cyanohydrins 0.9 ± 0.2, HCN 1.2 ± 0.2); dietary exposure to cyanogenic glycosides 203–669 μmol or 4–9 μmol/ kg bw	At 0 h (after eating porridge): 10 ± 4 μmol/24 h; after 5 days: 168 μmol	—	—	—	At 0 h: 0 μmol; at 24 h: 102 μmol/24 h; at 48 h: 124 μmol/24 h

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (μmol/l)	Serum thiocyanate Mean ± SD or range (μmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (μmol/l)
United Republic of Tanzania[16]	NS	Schoolchildren 10–18 years	193	Low-income area	High cassava consumers (usually sweet variety); 97% consumed cassava in previous week; mean of 4.8 cassava meals per week	36 ± 3	—	—	—	18 ± 1
United Republic of Tanzania[17]	1991	Women aged 15–45 years	217	Area where goitre is prevalent (total goitre in 72.8% and visible goitre in 13.3%)	Ugali made from cassava roots consumed twice per day by 80% of respondents to FFQ and at least once per day by 98%; cassava leaves and cabbage rarely consumed	128 (62% < 100; 38% > 100)	—	—	—	—

Table 3 (contd)

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (μmol/l)	Serum thiocyanate Mean ± SD or range (μmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (μmol/l)
Zaire (now Democratic Republic of the Congo)[18]	NS	NS	24	Goitre frequency 1.5%	High consumers of insufficiently processed cassava; 96% consuming cassava in previous 24 h	7.24 ± 1.09 mg/24 h	—	—	—	—
			152	Goitre frequency 12.5%	High consumers of insufficiently processed cassava; 93% consuming cassava in previous 24 h	5.88 ± 0.31 mg/24 h	—	—	—	—

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (µmol/l)	Serum thiocyanate Mean ± SD or range (µmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (µmol/l)
			140	Goitre frequency 76.8%	High consumers of insufficiently processed cassava; 91% consuming cassava in previous 24 h	10.75 ± 0.61 mg/24 h	—	—	—	—
Zaire (now Democratic Republic of the Congo)[19]	NS	NS	NS	Goitre frequency 60%	Consumers of cassava root dish 13.5 mg HCN/kg and cassava leaf dish 8.5 mg HCN/kg	2.57 ± 0.10	1.24 ± 0.03	—	—	—
				Goitre frequency 34%	Consumers of cassava root dish 3.5 mg HCN/kg	1.32 ± 0.11	0.42 ± 0.04	—	—	—

Table 3 (contd)

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (μmol/l)	Serum thiocyanate Mean ± SD or range (μmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (μmol/l)
				Goitre frequency 17%	Consumers of cassava root dish 3.5 mg HCN/kg; high consumers of fish	1.60 ± 0.07	0.78 ± 0.04	—	—	—
Zaire (now Democratic Republic of the Congo)[20]	1998	Schoolchildren	31	Konzo affected	95% were cassava consumers; ate cassava flour soaked for ≤3 days	757 ± 85	—	—	5.8 ± 0.9	—
		Adults	77	Konzo affected	95% were cassava consumers; ate cassava flour soaked for ≤3 days	904 ± 64	307 ± 14	—	—	—

Country[a]	Year	Population group	Number of subjects	Key population group features	Relevant consumption information	Biomarker[b]				
						Urinary thiocyanate Mean ± SEM (µmol/l)	Serum thiocyanate Mean ± SD or range (µmol/l)	Urinary total sulfur Mean (mol/mol creatinine)	Urinary inorganic sulfate Mean ± SEM (mmol/l)	Urinary linamarin Mean (µmol/l)
			46	Not konzo affected	99% were cassava consumers; ate steamed/ boiled paste from cassava roots soaked for ≥3 days	50 ± 7	—	—	5.0 ± 0.6	—
Sweden (controls)		Schoolchildren	17	NS	0% consumers of cassava	31 ± 4	—	—	22.3 ± 1.8	—

FFQ, food frequency questionnaire; NS, not specified; SD, standard deviation; SEM, standard error of the mean

[a] References are as follows: 1. Tylleskär et al., 1994; 2. Peterson et al., 1995; 3. Banea-Mayambu et al., 2000; 4. Hernandez et al., 1995; 5. Chiwona-Karltun et al., 2000; 6. Cardoso et al., 2004; 7. Cliff et al., 1985; 8. Ministry of Health Mozambique, 1984; 9. Cliff et al., 1997; 10. Cliff et al., 2011; 11. Ernesto et al., 2000; 12. Ernesto et al., 2002a; 13. Okafor, Okorowkwo & Maduagwu, 2002; 14. Onabolu et al., 2001a; 15. Carlsson et al., 1999; 16. Mlingi et al., 1998; 17. Mlingi et al., 1996; 18. Delange, Ekpechi & Rosling, 1994; 19. Lagasse et al., 1980; 20. Tylleskär et al., 1992.

[b] Units are as given in column headings unless otherwise specified.

[c] Estimated via equations.

goitre. These effects are often seen when cassava is the principal source of calories and its consumption is associated with protein and vitamin deficiencies. The toxic effects of cyanide on the thyroid are due to thiocyanate and seem to occur only in the presence of iodine deficiency.

In the previous evaluation by the Committee (Annex 1, reference *101*), it was considered that the association between chronic exposure to cyanogenic glycosides and neurological diseases based upon epidemiological observations was confounded by nutritional deficiencies, and a causal relationship could not be definitively established. Furthermore, because of a lack of quantitative toxicological and epidemiological information, a safe level of intake of cyanogenic glycosides could not be estimated. However, it was concluded that an HCN level of up to 10 mg/kg, as specified in the Codex standard for edible cassava flour (Codex Standard 176-1989), was not associated with acute toxicity.

Since the last JECFA assessment, other agencies and regulatory bodies have assessed the potential risk of cyanogenic glycosides in food. In 2004, the European Food Safety Authority (EFSA) considered the available data as not adequate to establish a numerical no-observed-adverse-effect level (NOAEL) or TDI for chronic exposure in humans, and a similar conclusion regarding cyanogenic glycosides in cassava and bamboo shots was reached by Food Standards Australia New Zealand (FSANZ, 2004b). The EFSA report noted that the level of 10 mg HCN per kilogram cassava flour, when applied to a consumption of 200 g/person, would lead to an estimated HCN intake of about 30 µg/kg bw for a 60 kg adult. Limited data from the United Kingdom and Norway showed that the high (97.5th percentile) exposures to HCN from its use in flavouring ingredients were 3.6 and 5.4 µg/kg bw, respectively, unlikely to give rise to acute toxicity.

The International Programme on Chemical Safety (IPCS, 2004) stated that long-term consumption of cassava containing high levels of cyanogenic glycosides, usually when constituting the principal source of calories and associated with malnutrition and protein and vitamin deficiencies, has been associated with neurological diseases involving tropical ataxic neuropathy and endemic spastic paraparesis. Although daily cyanide exposure has been crudely estimated to be 15–50 mg/day in endemic areas, owing to the limitations of data on exposure, which is likely to be variable, and the potential impact of confounders, such as general malnutrition, low protein content of the diet and iodine status, the available data did not provide meaningful information on a dose–response relationship for cyanide. For acute exposure, it referred to the then current edition of the WHO Guidelines for Drinking-water Quality, which derived a TDI of 0.012 mg/kg bw, applying an uncertainty factor of 100 to a lowest-observed-adverse-effect level (LOAEL) of 1.2 mg/kg bw in a study in pigs showing functional thyroid changes.

The Committee of Experts on Flavouring Substances of the Council of Europe (CoE, 2005) established a temporary maximum daily intake of 0.023 mg/kg bw based on a NOAEL of 4.5 mg/kg bw from a 13-week study showing adverse reproductive effects on male rats, applying a safety factor of 200. As a reference for human exposure, the Council of Europe used the daily cyanide exposure of 0.19–0.37 mg/kg bw estimated in populations from rural Democratic Republic of the Congo (formerly Zaire) affected by epidemic spastic paraparesis (konzo). The temporary

maximum daily intake is a factor of 10 below the lowest estimated exposure level of 0.19 mg/kg bw leading to neurological disorders in cassava-eating populations. This safety margin was considered adequate, as konzo is associated with sulfate deficiency, making it less likely to occur in adequately nourished populations. The United States Agency for Toxic Substances and Disease Registry (ATSDR, 2006) derived an intermediate oral minimal risk level of 0.05 mg/kg bw for cyanide based on a NOAEL of 4.5 mg/kg bw per day from the same 13-week study, applying an uncertainty factor of 100.

More recently, WHO published a background document for the development of a WHO drinking-water guideline for cyanide (WHO, 2009a) as well as one for the development of a WHO drinking-water guideline for cyanogen chloride (WHO, 2009b), which was derived based on cyanide. It was noted that if cyanogenic glycosides are not removed from cassava tubers by pretreatment, HCN can be liberated from the cyanogenic glycosides after ingestion and hydrolysis by the glycosidases[1] of the intestinal microflora and, to a lesser degree, those of the liver and other tissues. Based on a reported NOAEL for cyanide of 4.5 mg/kg bw per day from the subchronic study described above in which rats were exposed to sodium cyanide through drinking-water, a TDI for cyanide of 0.045 mg/kg bw per day was derived. It was considered acceptable to allocate 40% of the TDI to drinking-water for short-term exposure to allow for exposure to cyanogenic glycosides in food.

Below, the most relevant results are reviewed from works published after the last JECFA meeting, dealing with acute effects of cyanide and the assessment of chronic exposure to cyanogenic glycosides in cassava-eating populations, mainly concerning its potential causal role in konzo, but also other neurological disorders. Some of these works have already been reviewed in the above-mentioned reports.

(a) Acute toxicity

The acute oral toxicity of HCN in humans has previously been reviewed by this Committee (Annex 1, reference *102*). This section describes studies on the acute toxicity associated with exposure to both cyanide and cyanogenic glycosides in humans, as the acute toxicity of cyanogenic compounds is due to its conversion to cyanide.

Acute exposure to cyanide has occurred most frequently by the oral route from attempted suicides and homicides by ingestion of sodium or potassium cyanide. However, accidental poisonings due to cyanide intoxication may arise from eating apricot kernels, almond seeds, chokecherries and other stone fruit kernels, as well as inadequately prepared cassava or cassava products with high concentrations of cyanogenic glycosides (EFSA, 2004; IPCS, 2004; CoE, 2005). Cyanide causes a decrease in the utilization of oxygen in the tissues, producing a state of histotoxic anoxia. This occurs through inactivation of tissue cytochrome oxidase by cyanide, which combines with Fe^{3+}/Fe^{2+} contained in the enzyme. The clinical signs of

[1] Glycosidase is often used interchangeably with glucosidase, which tends to be used more frequently.

acute toxicity are well described and are dominated by central nervous system and cardiovascular disturbances, including headache, dizziness, mental confusion, stupor and cyanosis with twitching and convulsions, followed by coma. Sequelae of severe acute cyanide exposure have also been reported to include Parkinson-like syndromes and cardiovascular signs of delayed post-hypoxic myocardial lesions, as well as neuropsychiatric manifestations similar to those observed with post-hypoxic post–carbon monoxide encephalopathy (Uitti et al., 1985; Carella et al., 1988; Kadushin et al., 1988). If the HCN level exceeds the limit an individual is able to detoxify/tolerate, death may occur due to cyanide poisoning.

No specific gross or histopathological lesions are seen following acute cyanide poisoning, and there are no autopsy characteristics that are considered pathognomonic for death from cyanide poisoning. The toxic effects associated with acute exposure to cyanide in humans and animals are generally similar and are believed to result from inactivation of cytochrome oxidase and inhibition of cellular respiration during the terminal reaction of the electron transport chain (Annex 1, reference *102*).

The lowest published lethal dose of HCN in humans after oral administration is 0.57 mg/kg bw (RTECS, 2004). From several reports, the acute lethal oral dose of potassium cyanide for humans is reported to be 0.5–3.5 mg/kg bw (0.48–3.37 mg/kg bw as cyanide). Based on analyses of cyanide contents in tissues and in gastrointestinal tract contents among fatal oral poisoning cases and comparative kinetics with dogs, it has been estimated that death occurred after absorption of an average HCN dose of 1.4 mg/kg bw; the lowest fatal absorbed HCN dose was 0.54 mg/kg bw. In most poisoning cases, a large part of the ingested cyanide remained in the gastrointestinal tract; thus, using the dose ingested as an indicator of the lethality of cyanide may be misleading (IPCS, 2004).

A systematic review of confirmed cases of paediatric cyanide poisoning included cases of poisoning from inhalation of HCN in fire smoke, ingestion of household and workplace substances containing cyanogenic compounds, ingestion of cyanogen-containing foods and other sources of HCN, such as the hypotensive agent nitroprusside. The report included 14 episodes of poisoning by cyanogenic foods; 11 were due to ingestion of cassava or cassava products, and the remaining 3 cases were caused by ingestion of cycad seeds. The age range of affected subjects was 2.5–17 years; 3 of them died as a consequence of HCN poisoning, and the remaining 11 survived with no sequelae. Vomiting was the initial symptom in 10 children (all of them survived), and another survivor was asymptomatic. One of the three children who died presented with abdominal cramps, nausea and diarrhoea, another one with dizziness, headache and vomiting progressing to shock with acute renal failure, and the third one with coma. Symptoms started between 5 and 10 hours after ingestion. Blood concentrations of HCN were reported in five cases; two children who survived had blood HCN concentrations of 0.56 mg/l (19 hours after ingestion) and 0.32 mg/l (23 hours after ingestion). In two children who died, the blood levels were 0.85 and 1.35 mg/l (no time from ingestion was reported). In another case, the reported blood concentration was 4 mg/l, but the authors suggested that this value was erroneously high (Geller et al., 2006).

Cyanogen poisoning was also reported for a previously healthy 41-year-old woman who chewed and swallowed approximately 30 apricot kernels (estimated 15 g) (Suchard, Wallace & Gerkin, 1998). Within 20 minutes, she developed generalized weakness and numbness. After recognition of cyanide intoxication at the hospital emergency department, cyanide antidote treatment was administered, and the patient was discharged in stable condition on the second hospital day. Five hours after apricot kernel ingestion, the whole blood cyanide level was 43 µmol/l (1.1 mg/l), and the plasma thiocyanate level was 448 µmol/l (26 mg/l). The cyanide content of apricot kernels has been reported to be 2.92 mg/g, with a range from 0.122 to 4.09 mg/g; thus, the potential ingested dose of cyanide was approximately 44 mg (0.73 mg/kg bw for a 60 kg adult). The continuing sale of apricot kernels as health food is believed to be related to the inappropriate use of laetrile as a cancer treatment; although the compound trademarked as Laetrile is a monoglucoside derivative of mandelonitrile, most samples of laetrile products contain amygdalin instead. Interestingly, in a previous review (Geller et al., 2006), four cases of cyanide poisoning were reported due to ingestion of Laetrile tablets or a Laetrile enema in children; a 11-month-old girl presented with coma and died in spite of treatment, whereas the other three survived without sequelae. No blood cyanide levels were reported.

Patients with histologically confirmed cancer, who had not responded to standard treatments, were treated with Laetrile (amygdalin). Among the 179 patients, 165 were given Laetrile in 21 daily intravenous injections at a daily dose of 4.5 g/m^2 body surface, followed by oral administration of 0.5 g 3 times a day (25 mg/kg bw per day for a 60 kg adult), whereas 14 received a high-dose regimen of 7 g/m^2 intravenously and 0.5 g orally 4 times per day (33 mg/kg bw per day). These oral doses correspond to 1.5–2 mg of cyanide equivalents per kilogram body weight. All subjects also received vitamins A, C, E and B complex and pancreatic enzymes. Whole blood cyanide was determined after completion of intravenous treatment, 48 hours after initiation of oral therapy and at every subsequent evaluation. Oral therapy was discontinued if the blood cyanide level was found to be 3 µg/ml or higher at any time. Among the 134 patients on oral treatment evaluated, 30% had nausea, 17% vomiting, 10% dizziness, 8% headache and 5% obtundation. These effects subsided when therapy was discontinued. Levels of cyanide in blood increased rapidly and stabilized after 48 hours; 69 out of 105 monitored patients had levels below 1 µg/ml, and 3 had levels higher than 3 µg/ml (maximum 3.7 µg/ml). The average level was approximately 0.6 µg/l, with 5th and 95th percentiles of 0.1 and 2.3 µg/ml, respectively (Moertel et al., 1982).

An outbreak of acute intoxication from consumption of insufficiently processed cassava was documented in the United Republic of Tanzania. In three villages from the Massai District, 35 households were selected to collect information about cassava consumption and processing, occurrence of acute intoxications and measurements of thiocyanate. Plasma and urine were collected from 95 subjects, as well as specimens of cassava flour, both in a normal year and in a period after drought and food shortage, when cassava was the only crop that survived. During the food shortage period, the normal method for processing cassava was replaced by two shortcut methods, much less efficient in reducing the cyanogen contents in cassava flour. As reference, plasma and urine thiocyanate levels were measured in 201 subjects from a region where cassava consumption was rare.

Of the 35 households interviewed, 80% confirmed that most family members had suffered acute intoxications on one or several occasions during the food shortage period. Six cases were admitted to two hospitals in the district. A 10-year-old boy admitted unconscious after sudden onset of symptoms following consumption of bitter cassava died after several hours at the hospital, due to unavailability of antidotes; the remaining five cases recovered within 24 hours. Plasma thiocyanate levels were on average 355 µmol/l in subjects during the cassava drought year and 28 µmol/l in subjects from the control village. Mean urinary thiocyanate levels in cassava-eating villages were 1120 µmol/l during a drought year and 68 µmol/l in a normal year; in the control village, the mean urinary thiocyanate level was 7 µmol/l. Cyanogen content was measured in cassava flour in a normal year and in cassava flour processed by means of two shortcut methods during the shortage period. The mean HCN level was similar for the three samples; the glycoside level was similar also for the most often used shortcut method and the usual processing method during a normal year. However, the average level of cyanohydrin was higher in cassava flour processed by the shortcut methods (48 and 15 mg HCN equivalents per kilogram for the two methods, respectively), compared with 7 mg/kg measured in cassava flour in a normal year (Mlingi, Poulter & Rosling, 1992).

Finally, in a draft for the fourth meeting of the Foodborne Disease Burden Epidemiology Reference Group (FERG, 2010), 22 reports of acute cyanide poisoning were reviewed. Three types of poisoning were identified: first, sporadic accidents mostly in children; second, epidemics during droughts, sometimes in areas where cassava is newly introduced; and third, outbreaks associated with epidemics of konzo. The report suggested that acute poisoning may go unreported because it is common, is non-fatal and occurs mostly in remote areas. It may also truly be rare, despite evidence of considerable cyanide exposure from consumption of cassava. The ingestion of porridge rich in cyanogenic glycosides and acetone cyanohydrin will result in a gradual release of cyanide in the gut over many hours. When the detoxification rate is similarly reduced because of low sulfur availability, the blood cyanide level will rise and fall slowly. These two factors will lead to continuously high, but sublethal, blood cyanide levels, which will rarely reach the level producing clinical symptoms or fall to normal levels.

(b) Toxicity from long-term exposure to cyanogenic glycosides in cassava-eating populations

Long-term consumption of cassava containing high levels of cyanogenic glycosides, usually when cassava constitutes the main source of calories, has been associated with neurological diseases involving endemic spastic paraparesis (konzo) and tropical ataxic neuropathy. In areas with low iodine intake, development of hypothyroidism and goitre, sometimes accompanied by the neurological diseases, has also been linked to cassava consumption.

(i) Konzo (spastic paraparesis)

Konzo is an upper motor neuron disease characterized by irreversible but non-progressive symmetric spastic paraparesis that has an abrupt onset. It mostly affects children and women of childbearing age. In most epidemics, its occurrence

has been associated with high and sustained exposure to cyanogens at sublethal concentrations from cassava or cassava flour in combination with a low intake of sulfur-containing amino acids (Annex 1, references *101* and *102*). The disease was first described in 1938 and was named after the local designation used by the population in the Democratic Republic of the Congo (then known as Zaire) meaning "tied legs", a description of the resulting spastic gait. Since the previous JECFA evaluation, epidemic outbreaks of konzo have been reported in the Democratic Republic of the Congo, Mozambique, the United Republic of Tanzania and the Central African Republic.

Konzo outbreaks in the Democratic Republic of the Congo. In the Bandundu Region, konzo appeared in the mid-1970s, and new cases continued to occur during the 1980s. Research was undertaken in 1988–1990 to examine the potential role of cyanogens from cassava in konzo outbreaks in the region. A field survey was conducted in 40 randomly selected households in affected villages (south of Kasai river) and 167 schoolchildren from four representative villages north of Kasai river where no konzo cases had been reported or found when children were screened. Urine and urinary specimens were obtained from 77 adults and 31 children from affected households and 46 children from the unaffected villages. Samples of cassava flour obtained from cassava roots processed according to traditional methods and shortcut methods used when konzo cases had occurred were collected to measure the cyanogen content. During the field survey in konzo-affected villages, three new cases were identified, from which blood specimens were taken; 23 healthy subjects from affected villages were randomly selected as controls. Both the affected and unaffected populations had a very high bitter cassava consumption, but the methods by which they processed the foods differed: the traditional method used in unaffected villages involves soaking the roots for at least 4 days and reduces the cyanogens to negligible concentrations, whereas the shortcut method used in affected villages, in which roots are soaked for a much shorter time, leaves considerable quantities of cyanohydrins in the prepared flour. Flours consumed by households in affected villages contained a mean cyanide equivalent concentration of 32 mg/kg (44% from glycosides and 42% from cyanohydrins). Adults in the affected population had mean urinary and serum thiocyanate levels of 904 and 307 µmol/l, respectively (52.5 and 17.8 mg/l, respectively). As for children, the mean urinary thiocyanate concentrations were 757 µmol/l in the affected population and 50 µmol/l in the unaffected ones; as a reference, the mean level was 31 µmol/l in 17 Swedish schoolchildren. The corresponding concentrations of urinary sulfate were 5.8, 5.0 and 23.3 mmol/l, respectively, yielding mean thiocyanate:sulfate molar ratios of 0.20, 0.01 and 0.002. The three patients with konzo were boys aged 4, 7 and 14 years, examined, respectively, 18, 36 and 90 hours after the onset of disease. The three cases, but only 2 out of 23 controls, had blood cyanide levels above 4 µmol/l (in a range 20–60 µmol/l); these 2 controls had serum thiocyanate levels well above 400 µmol/l, whereas the konzo patients had thiocyanate concentrations below 400 µmol/l. The mean level of serum thiocyanate in Swedish non-smoking adults from a study by Lundquist et al. (1979) was 42.5 µmol/l, and the 95th percentile level (used as a reference level) was 76 µmol/l. As cassava flour consumption in these populations is above 0.5 kg/day (and considering the 32 mg of cyanide equivalents per kilogram found in cassava flour),

the authors considered that the potential daily cyanide exposure was approximately 0.5–1 mmol of cyanide (13–26 mg). These figures of estimated daily intake were used to derive an indicator of cyanide exposure of 0.19–0.37 mg/kg bw for a 70 kg adult. According to the authors, the low urinary sulfate level is a consequence of low sulfur levels in these cassava-eating populations, and the high thiocyanate:sulfate ratio in the affected population indicates that a substantial part of the available sulfur was used for cyanide detoxification. Exposure to cyanide below the maximum cyanide to thiocyanate conversion rate results in a moderate increase in blood cyanide to 0.4–4 μmol/l (accumulation level); however, higher exposure results in much higher blood cyanide concentrations. The fact that the three patients had blood cyanide concentrations far above the accumulation level indicates a potential causal role of cyanide in konzo. The authors hypothesized that if the high cyanide concentrations inducing konzo result from the combined high cyanide exposure and decreased conversion rate because of deficient sulfur substrate, this would explain the lower urinary thiocyanate levels observed in konzo cases compared with controls. All patients and controls were negative for antibodies of human T-cell lymphotropic virus-1 (HTLV-1) and human immunodeficiency virus (HIV-1, HIV-2) (Tylleskär et al., 1992).

As a consequence of the previous study, a case–referent study was carried out in five of the most konzo-affected villages in the Bandundu Region in 1990. In the study population of 1301 inhabitants, 63 konzo cases were identified. Six were excluded because they lived at distant isolated settlements; thus, 57 cases living in villages were included. For each case, one referent was selected, matched by sex, age at onset and village. Of the 57 konzo patients, 24 had their onset at 3–15 years, 27 as women of childbearing age and 3 as adult males. One of them had the onset in 1983, 2 in 1984, 12 in 1985, 18 in 1986, 9 in 1987, 6 in 1988 and 5 in 1990; 46 of them (81%) had the onset during the dry season. Dietary information collected referred both to the previous 3 months and to the 3-month period preceding the onset of disease. A negative association (although non-significant) was observed for fish consumption (odds ratio [OR] = 0.31 for fish consumption at least weekly). As for cassava consumption, the most important finding was related to the methods of processing, involving 2 or 3 nights of soaking; the shorter the soaking time, the lower the reduction in cyanogens from cassava roots. The 2 nights' soaking method was significantly associated with the risk of konzo (OR = 11, 95% confidence interval [CI] = 1.7–73). Among those eating cassava soaked for 2 nights, there was a strong dose–response association: compared with consumers with cassava ingestion less than once per week, those who consumed cassava at least weekly had an OR of 18, and those consuming cassava at least daily had an OR of 61 (Tylleskär et al., 1995).

An ecological study was carried out in Paykongila (Bandundu Region), where 78 cases among 1400 inhabitants in eight savannah villages were detected in 1990. Within the three most affected savannah villages, a dietary interview was gathered from 105 members from 19 konzo-affected households and 150 members from unaffected households; furthermore, 160 members from 30 households from forest villages without reported konzo cases were also included as controls. Urine specimens were collected from 22 konzo patients and 57 members from konzo households, as well as from 116 controls from unaffected households in the savannah

villages and 103 controls from the forest villages. Cassava was consumed daily in almost all households, in both the savannah and the forest villages, according to the 24-hour food recall. It was consumed mainly in the form of a porridge known as luku in the local language, made from flour obtained by pounding soaked and dried roots. However, in the forest households, cassava flour was significantly more often (73% of households) mixed with maize flour, whereas this practice was very rare in the savannah villages. Average urinary levels of thiocyanate were high in all groups in the affected villages (konzo patients 563 µmol/l, other members 587 µmol/l, controls 629 µmol/l), whereas they were 241 µmol/l in forest villages. Inorganic sulfate excretion was low, ranging from 2.2 mmol/l in konzo patients to 3.8 mmol/l in controls from forest villages. However, urinary levels of linamarin were higher in subjects from affected households (632 and 657 µmol/l in konzo patients and unaffected members from the same households) than in subjects from unaffected households in savannah villages (351 µmol/l) and controls from the forest villages (157 µmol/l). The authors suggested that the association found between konzo and urinary linamarin levels raises the question as to whether linamarin may be an etiological factor apart from its contribution to general cyanide exposure (Banea-Mayambu et al., 1997).

In August 1996, an outbreak of konzo (initially diagnosed as poliomyelitis) was reported in a different region within the Bandundu Region. A total of 237 subjects met the case definition, for a prevalence of 2.4 per 1000 inhabitants. No quantitative analysis of the association with cassava consumption was provided. No change was reported in cassava processing over time, and the same variety of cassava was cultivated. Many people complained of decreasing yields of root crops over the previous decade; there were no reports of droughts, although this could not be confirmed, as no rainfall data were available. Finally, in 2004, a study was conducted in the Popokabaka rural zone in the Bandundu Region, belonging to the district of Kwango, where cases of konzo were first reported in 1938 (Diasolua Ngudi et al., 2011). Among 3015 individuals in the 487 households selected, 43 konzo patients were detected (prevalence 1.4%) in 33 households; two thirds of patients were under 15 years of age. The prevalence of konzo was associated slightly with consumption of cassava originating from the patients' own farmland as opposed to cassava obtained elsewhere. A daily median of 304 g (range 120–592 g) of cassava flour providing 4.5 MJ (range 1.8–8.7 MJ) and 7 g of protein, thus 0.10 g of methionine and 0.61 g of cysteine per person, was estimated in this population (Bonmarin, Nunga & Perea, 2002).

Konzo outbreaks in other African countries. The main features of the several outbreaks of konzo in Mozambique during the last 30 years were recently reviewed. The first case of konzo in Mozambique was reported in 1981, within a large epidemic in the northern Nampula province. Smaller epidemics were reported in the province through the 1980s, including the war period 1984–1992. In 1992–1993, a large epidemic occurred farther south in the province. In 2005, an outbreak occurred in another district of the province, and new cases continued to be reported in previously affected areas of Nampula (Ernesto et al., 2002b). In 2000, konzo emerged in Zambezia province, farther south. Epidemics have occurred mostly during agricultural crises, when the population has been dependent on a diet of insufficiently processed bitter cassava. Cassava is often processed by sun drying

for several weeks, which is an inefficient method. Heap fermentation, by pounding the roots, is also used; although the resulting flour has lower total cyanogenic potential, it is still unsafe when the initial cyanogenic glycoside concentration in the root is high. The average total cyanogenic potential is 40–46 mg HCN equivalents per kilogram flour (fresh weight) in normal years; in years of low rainfall, it increases to more than 100 mg HCN equivalents per kilogram. The mean urinary excretion of thiocyanate measured in patients with konzo ranged from 272 to 528 µmol/l; the concentrations in schoolchildren from affected areas was in the range of 219–351 µmol/l. In schoolchildren in a konzo-affected village, the mean concentration of inorganic sulfate in urine was 0.73 mmol/mol creatinine, compared with 1.34 and 1.36 mmol/mol creatinine measured in schoolchildren in the district capital and Swedish children (Cliff et al., 2011).

A recent paper reviewed the occurrence of konzo in the United Republic of Tanzania. The first konzo outbreak in the United Republic of Tanzania was reported in the drought-affected district of Tarime in Mara region in the north in 1985, with 116 verified cases. A second outbreak with three cases occurred in 1988 in Mtanda village, Massai district, Mtwara region, in southern United Republic of Tanzania, after reported frequent occurrences of acute intoxication following meals of cassava during a drought. The two most recent outbreaks of konzo occurred in 2001–2002 in Mbinga district, Ruvuma region, with 24 cases, and in 2002–2003 in Mtwara region, with 214 konzo cases. No formal quantitative assessment of related cyanogen exposure from cassava was provided; however, the authors concluded that every outbreak of konzo in the United Republic of Tanzania resulted from large cyanogen intakes from cassava during drought, which caused crop failure, food shortage and use of shortcut methods of processing cassava. Also during drought, it has been shown that the water-stressed cassava plant produces greatly increased amounts of linamarin, and cassava flour made from these roots contains much larger amounts of cyanogens than normal. Another factor that contributes to high cyanogen intake is the introduction of bitter cassava varieties from elsewhere (Mlingi et al., 2011).

An investigation was conducted in Baboua area, a sparsely populated highland area in the westernmost part of the Central African Republic, where the occurrence of konzo was suspected. In total, 16 cases were diagnosed, with the onset in a period from 1984 to 1992 and an age range from 4 to 18 years. No cases were found in four villages in another area with no suspected konzo, used as reference. Cassava was the almost exclusive staple in all seasons in both affected and unaffected families in the studied areas. All konzo patients had eaten cassava within the previous 24 hours. Interviews revealed that in the affected area, the cassava processing had been shortened during the previous decade by leaving out a fermentation step, whereas in villages from the unaffected area, cassava was consumed in equal amounts, but the processing was not shortened (Tylleskär et al., 1994).

The etiology of konzo associated with cyanogenic glycosides in cassava. In all populations where konzo epidemics have been reported, the diet described is uniformly similar: there were several weeks of almost exclusive, monotonous consumption of improperly processed bitter cassava roots. Apart from the high

cyanogen content, cassava roots are rich in calories, but they are markedly deficient in protein, particularly essential sulfur-containing amino acids, as well as some vitamins of group B. Furthermore, these populations often have other nutritional or metabolic deficiencies affecting vitamin A, zinc and iron.

The epidemiological association between cassava consumption and konzo, although mainly based upon studies at the aggregate level, is consistent. However, the etiological mechanisms of konzo remain unknown. High cyanide and low sulfur dietary intakes due to exclusive consumption of insufficiently processed bitter cassava roots have been proposed as the cause of konzo (Tylleskär et al., 1992). Cyanide is formed from precursor cyanogenic glycosides in cassava and absorbed in the gut; it is then converted to thiocyanate, a reaction that involves sulfur as a rate-limiting cofactor for the enzyme rhodanese. Because of the deficiency in sulfur-containing amino acids, exposure to cyanide beyond the conversion rate results in a high blood cyanide concentration, thought to play a causal role in konzo. This hypothesis has been criticized, because the excretion of thiocyanate, the main metabolite of HCN, is similar in cases and controls and because the patients and family members who did not succumb to the disease had been shown to have similar levels of cassava consumption. On the other hand, spastic paraparesis, the clinical hallmark of konzo, is not a known clinical manifestation of cyanide toxicity and has not been associated with cyanide exposure from any other source. Furthermore, the disease has not been induced in animals.

An alternative hypothesis has been suggested: that konzo is a consequence of a thiamine deficiency state (Adamolekun, 2010). The underlying mechanisms would be an inactivation of thiamine (vitamin B1) that occurs when the sulfur contained in thiamine is utilized for the detoxification of cyanide from inappropriately processed cassava. Cyanide is converted to thiocyanate using the sulfur of cysteine and methionine, the sulfur-containing amino acids from dietary proteins. However, rhodanese has a wide substrate specificity; therefore, sulfur compounds other than sulfur-containing amino acids may function as sulfur donors, and this may be the case when there is a dietary sulfur shortage because of low intake of protein and sulfur-containing amino acids. Thiamine is one of such sulfur compounds and is known to be rendered inactive when the sulfur of its thiazole moiety is combined with HCN. There has been shown to be a widespread thiamine deficiency in susceptible populations; thus, in populations with marginal thiamine deficiency, the cassava-induced inactivation of thiamine may lead to clinical syndromes. Some neurological manifestations common in konzo, such as spastic paraparesis, optic neuropathy and peripheral sensory neuropathy, are also manifestations of thiamine deficiency. Further support for the thiamine hypothesis is provided by recent evidence indicating that tropical ataxic neuropathy, the only other neurological disorder thought to be caused by cyanide intoxication from cyanogenic glycosides in cassava, may also result from a thiamine deficiency (Adamolekun, 2011).

Another unifying hypothesis attributes to nitriles the role of causative agents of konzo, tropical ataxic neuropathy and lathyrism; the latter is a clinical disease similar to konzo associated with consumption of grass pea (Llorens et al., 2011). The hypothesis is based upon increasing evidence, mainly from experimental studies, indicating that nitriles have a variety of neurotoxic effects. Nitriles are a large

group of compounds that includes β-aminopropionitrile, thought to be the potential neurotoxicant causing lathyrism. In the case of cassava, the acetone and butanone cyanohydrins released from hydrolysis of linamarin and latoaustralin, respectively, are nitriles. However, other nitriles could be generated during food processing or in the body. The neurotoxic potential of cassava nitriles remains largely unexplored.

Another hypothesis suggests that deficiencies of the sulfur-containing amino acids, methionine and cysteine, are major predisposing factors in both konzo and lathyrism, as they lead to a reduction in the biosynthesis of glutathione in the central nervous system (Nunn, Lyddiard & Perera, 2011). As already stated, cassava roots are poor sources of proteins and sulfur-containing amino acids, used for cyanide conversion to thiocyanate as sulfur donors. Thus, monotonous diets containing cassava with high cyanogenic glycoside content induce severe deficiency of both cysteine and methionine. These amino acids are precursors for the synthesis of glutathione in the central nervous system. Results from experimental studies suggest that sulfur-containing amino acid deficiencies may be associated with the neuropathology observed in konzo and lathyrism.

Finally, it has been postulated that thiocyanate, the main metabolite of cyanide, modulates amino-hydroxy-methyl-isoxazole-propionic acid receptor function (Spencer, 1999). Although amino-hydroxy-methyl-isoxazole-propionic acid receptors can be mediators of the motor neuron degeneration that underlies konzo and lathyrism, it would be difficult to explain the fact that in populations with konzo outbreaks, the urinary and blood concentrations of thiocyanate in unaffected subjects are similar to or even higher than those in konzo patients.

(ii) Tropical ataxic neuropathy

Tropical ataxic neuropathy is used to describe several neurological syndromes attributed to toxico-nutritional causes. The syndromes grouped as tropical ataxic neuropathy can differ widely in clinical presentation, natural history and response to treatment. Tropical ataxic neuropathy has occurred mainly in Africa, particularly Nigeria. The main clinical features of some of the syndromes include sore tongue, angular stomatitis, skin desquamations, optical atrophy, neurosensory deafness and sensory gait ataxia. Tropical ataxic neuropathy has been found associated with dietary cyanide exposure from the chronic monotonous consumption of foods processed from cassava, but the etiology of ataxic polyneuropathy is unknown (Annex 1, references *101* and *102*). In contrast with konzo, only a few studies have been published since the last JECFA evaluation on the association between tropical ataxic neuropathy and cassava consumption.

An ecological study was conducted to determine the relationship between intake of cassava foods and dietary cyanide load and tropical ataxic neuropathy in Nigerian communities. Three geographical areas were defined: area A (south-western Nigeria) included communities with endemic tropical ataxic neuropathy and high cassava consumption; area B (south-western Nigeria) included communities with low cassava consumption and absent tropical ataxic neuropathy; and area C included communities in northern Nigeria where tropical ataxic neuropathy had not been described and cassava was not the staple. In total, 1272 subjects were sampled, 238 in area A, 659 in area B and 375 in area C, for which history of

food eaten during the preceding week and spot urine samples were collected, and samples of cassava products were obtained from local markets. The mean intakes of cassava foods per person per week were 17 meals in area A, 10 meals in area B and 1 meal in area C. The geometric mean urinary thiocyanate concentration was 73 µmol/l in area A (95% CI = 66–80 µmol/l), 51 µmol/l in area B (95% CI = 48–54 µmol/l) and 17 µmol/l in area C (95% CI = 15–19 µmol/l). The mean cyanogen contents of cassava food samples were 16 mg HCN equivalents per kilogram dry weight for area A and 13 mg HCN equivalents per kilogram dry weight for area B. In 24 (86%) cassava food samples from area A and 32 (63%) samples from area B, the cyanogen content exceeded 10 mg HCN equivalents per kilogram dry weight. The presence of high dietary cyanide load outside the geographical area where tropical ataxic neuropathy has been described suggests that high dietary cyanide load is not exclusive to these communities. Two communities in south-western Nigeria have a higher dietary cyanide load than the communities where tropical ataxic neuropathy had been described (Onabolu et al., 2001b).

In Nigeria, where ataxic polyneuropathy was first described in endemic form in 1955, the majority of cases are clustered in a geographical area in the south-west; however, ataxic polyneuropathy occurs in sporadic, epidemic and endemic forms. An ecological study compared the occurrence of ataxic polyneuropathy in two communities from Oyo State in south-western Nigeria, one located in the endemic area (Ososa) and the other located outside the endemic area (Jobele). In total, 3428 subjects from Ososa and 1767 from Jobele, aged 10 years and above, were screened with the tandem walk test, and those who tested positive had a detailed clinical history taken and a clinical examination. In Ososa, 168 subjects were diagnosed with ataxic neuropathy (4.9% prevalence), with only three cases in Jobele (0.17%). A total of 60 and 151 subjects randomly selected from the high- and low-prevalence areas, respectively, provided blood and urine samples and were interviewed to assess cassava intake the previous week. The mean intake of all cassava foods was 10 meals per person per week in Ososa and 7 in Jobele. The intake of proteins was significantly lower in Ososa. No quantitative measurements were provided for blood and urine concentrations, but only the proportion of subjects above and below reference limits was determined in a sample of healthy adults. The concentration of thiocyanate in plasma and the excretion of inorganic sulfate were significantly lower in the high-prevalence area. The concentrations of glutathione, cysteine and γ-glutamylcysteine in the plasma and urinary thiocyanate levels were within the same range in both areas. The amount of cyanogenic compounds in the foods prepared by the method used to process cassava root in Jobele was higher than that in food processed by the reference method used in most communities in Nigeria where exposure to cyanide from cassava foods has been observed to be low. According to the authors, the finding that the exposure to cyanide from cassava foods is higher in the low-prevalence area suggests that exposure to cyanide from cassava foods may not have a direct role in the causal mechanism of ataxic polyneuropathy. In contrast, the excretion of inorganic sulfate, a biomarker of protein intake, is significantly lower in the high-prevalence area; it is therefore possible that low intake of protein is a proxy for a specific deficiency that is yet to be identified (Oluwole et al., 2002).

Following the previous work, an incidence study was carried out in Ososa, the high-prevalence community in the endemic area. A total of 3167 subjects aged 10 or above who were not diagnosed as having ataxic polyneuropathy or other neurological illness formed a cohort that was followed for 2 years. At the end of the follow-up, the cohort was screened with a tandem walk test, a full medical history and a history including neurological examination. After examination, 40 subjects among the 271 who screened positive to the test were diagnosed with ataxic neuropathy (incidence 64 per 10 000 person-years). Within healthy subjects, 120 were randomly selected as controls, and both cases and controls were interviewed to assess food history; a blood sample was obtained from 23 cases and 60 controls. There was no statistical difference in the intake of all cassava foods between cases and controls. Twelve cases (52%) compared with 24 controls (40%) had levels of thiocyanate above the reference limits (66 µmol/l). Exposure to cyanide was not significantly different between cases and controls. The concentrations of cysteine, γ-glutamylcysteine and glutathione in plasma were not different in cases and controls. The excretion of inorganic sulfate was higher (but not statistically significant) among cases. According to the authors, the finding of similar levels of thiols in cases and controls does not suggest deficiency of substrates needed to detoxify cyanide to thiocyanate in ataxic polyneuropathy (Oluwole et al., 2003).

Between 2000 and 2002, a study was carried out in a tertiary referral centre in the central area of Kerala, south India. Forty consecutive patients were prospectively recruited; they manifested both symmetrical sensory polyneuropathy and sensory gait ataxia; overt causes of neuropathies, such as diabetes, nutritional deficiency, toxins, porphyria, paraneoplastic syndrome, vasculitis and chronic inflammatory polyneuropathy, were excluded. Of the 40 patients, 12 were males and 28 females, with a median age of 28 years. Thirty-two patients gave a history of poor dietary intake in which the staple food was rice and tapioca, eaten at least twice a day. Many of the patients could not afford eggs, milk, meat or fish: meat was consumed once a month, and fish intake was once a week. Thiocyanate levels were measured in plasma, urine and sural nerve biopsy and compared with those from control samples taken from healthy individuals from the same locality, usually from the same household. The plasma levels of thiocyanate were 91 and 26 µmol/l for cases and controls, respectively; the corresponding values were 2.0 and 0.4 µmol/24 hours in urine and 28.7 and 6.0 µmol/g in the sural nerve. All the differences were statistically significant. The serum vitamin B12 level was normal in all the tested patients. According to the authors, the significantly higher thiocyanate levels in serum, urine and sural nerve biopsy specimens in cases compared with control subjects point to potential chronic cassava neurotoxicity, either alone or in association with poor nutrition, in the pathogenesis of tropical ataxic neuropathy in these patients (Madhusudanan et al., 2008).

(iii) Other neurological effects

A high proportion of apparently healthy children with ankle clonus had been reported in communities affected by konzo in Mozambique; it was attributed to subclinical damage from cyanide exposure. In 1993, a study was carried out in districts that had registered konzo cases during the previous year. Overall, 397 schoolchildren (mean age 10 years) were surveyed, and a prevalence of ankle

clonus of 12.3% was observed. Urine specimens were available for 131 children, including 16 with ankle clonus. Geometric mean concentrations in the overall sample were as follows: thiocyanate, 163 µmol/l; linamarin, 60 µmol/l; and inorganic sulfate, 4.4 mmol/l. The geometric mean concentration of thiocyanate in children with clonus was 244 µmol/l, significantly higher than the 154 µmol/l among those without clonus. The prevalence of clonus increased as the concentration of thiocyanate increased. Clonus in otherwise healthy subjects has also been reported from lathyrism areas in India. Thiocyanate excretion was higher among cases with clonus than among controls, but the overall mean was lower than the levels observed in konzo areas. The low geometric mean concentration of inorganic sulfate in urine is similar to the arithmetic mean concentrations measured in konzo areas, suggesting potential sulfur-containing amino acid deficiency (Cliff et al., 1999).

In 1988, a medical centre in Dar es Salaam in the United Republic of Tanzania reported 200 patients with sudden loss of central vision; the initial diagnosis was optic nerve and macular atrophy of unknown origin. Research was carried out on 20 such patients (8 males, 12 females, 11–39 years of age) to assess cyanide exposure. They were examined within 12 weeks of the onset of visual loss. Neurological and/or sensory signs were reported in six patients, and three showed a combination of severe neurological and audiological signs. The mean plasma concentration of thiocyanate was 23.6 µmol/l in patients, 15.9 µmol/l in relatives and 18.9 µmol/l in controls (staff members). The respective levels of blood cyanide were 0.09, 0.09 and 0.01 µmol/l. These differences were not significant. Two of the three subjects with severe neuro-audiological impairment had thiocyanate levels of 45.6 and 54.9 µmol/l. Although most patients presented only with optic atrophy, the presence of other neurological signs suggests polyneuropathy. Optical involvement is rare in konzo, whereas optic atrophy and perceptive deafness are common in tropical ataxic neuropathy. Levels of blood cyanide and thiocyanate were lower than those reported in patients with konzo and tropical ataxic neuropathy; however, the thiocyanate concentrations in the two patients with severe neurological and audiological loss were similar to levels found in patients with tropical neuropathy without ataxia. It remains questionable whether these patients had a nutritional neuropathy caused exclusively by chronic cyanide poisoning (van Heijst et al., 1994).

(iv) Goitre and thyroid function

Studies in African countries have established that goitre and cretinism due to iodine deficiency can be considerably aggravated by a continuous dietary exposure to cyanide from insufficiently processed cassava. This effect is caused by thiocyanate, which is similar in size to the iodine molecule and interferes with uptake of iodine into the thyroid gland. High thiocyanate levels, which can occur after exposure to cyanide from cassava, can affect the gland only when the iodine intake is below 100 mg/day, which is regarded minimal for normal function (Annex 1, references *101* and *102*).

A study was carried out in an area with endemic iodine deficiency disorders in western United Republic of Tanzania (Mlingi et al., 1996). In total, 217 women aged 15–44 years were selected; they were interviewed with respect to their cassava consumption history, provided urinary specimens and were examined

for the presence of goitre. Cassava flour obtained from processing fresh cassava roots in different ways was used to measure the cyanogen content. Total and visible goitre rates were 72.8% and 13.3%, respectively. The porridge made from processed cassava roots (ugali) was consumed twice daily by the majority (80%) in both dry and rainy seasons and at least once daily by 98%. The mean urinary iodine level was 4.55 µg/dl (median 3.6 µg/dl), and the mean urinary thiocyanate level was 128 µmol/l (median 70 µg/dl). The mean iodine:thiocyanate ratio was 35.8 µg/mg (median 9.4 µg/mg, range 0.2–1971 µg/mg). The prevalence of goitre was significantly associated with a urinary iodine level below 10 µg/dl (88% versus 77% in women with and without visible goitre). Although women with goitre had higher urinary thiocyanate levels and lower iodine:thiocyanate ratios, the difference was not significant. The increase in urinary thiocyanate levels observed in the population does not prove that thiocyanate aggravated the severity of the iodine deficiency disorders. The iodine:thiocyanate ratio was relatively high on average (35.8 µg/mg); however, the very skewed distribution of the ratios makes the use of a mean value unsatisfactory. Overall, 19% of women had a ratio below the threshold of 3 µg/mg (22% among those with goitre, 10% among those without goitre). This suggests that some individuals may have an interference of iodine handling in the thyroid by the pseudo-halide thiocyanate, although the association between low iodine:thiocyanate ratio and goitre occurrence was not significant in this study.

A cross-sectional study assessed the potential role of determinants of iodine deficiency disorders (other than iodine) in Ethiopia (Abuye, Berhane & Ersumo, 2008). Populations were sampled from five randomly selected regional states (two from the north and three from the south). Information on frequency of cassava consumption in households was collected, as well as urine samples from all children included in the study. The total goitre incidence in all regional states was greater than 20% in both children and mothers, an indication of severe to moderate iodine deficiency. Cassava consumption was associated with goitre rates in both children and mothers (OR = 1.99 and 2.00, respectively, both statistically significant). Within the states, the association persisted in two of them, but not in the third. Urinary iodine excretion ranged from 3.8 to 7.5 µg/dl. Besides regional differences, the level in all regional states was below 10 µg/dl, used as an indicator of iodine deficiency.

3. BIOLOGICAL DATA: CYANIDE

3.1 Biochemical aspects

3.1.1 Absorption, distribution and excretion

The absorption, distribution and excretion of HCN were extensively reviewed previously by the Committee (Annex 1, reference *102*). In brief, it was reported that HCN is readily absorbed after oral administration and rapidly distributed in the body through the blood. At the physiological pH of the stomach, cyanide will predominantly form HCN, which can rapidly penetrate mucous and cell membranes. Limited data are available on oral absorption of cyanide in humans. Following oral exposure to cyanide in humans, tissues reported to contain cyanide include the

liver, brain, spleen, blood, kidneys and lungs (Gettler & Baine, 1938; Ansell & Lewis, 1970). The concentration of cyanide is higher in erythrocytes than in plasma, as it is known to bind with iron in both methaemoglobin and haemoglobin present in erythrocytes (approximately 99% of an absorbed dose). Cyanide is primarily excreted in the urine as thiocyanate following oral exposure, with small amounts excreted through the lungs.

(a) Rats

In a 13-week toxicity study, male Sprague-Dawley (Tif: RAI f, SPF) rats (26–40 per group) received potassium cyanide in their drinking-water 7 days/week at doses of 40, 80 or 140/160 mg/kg bw per day. The control group received tap water. The behaviour and mortality of the animals were checked daily; body weight and feed consumption were determined once a week. Cyanide and thiocyanate concentrations were determined in blood, which was collected every 2 weeks, and in urine, which was collected over a 16-hour period during weeks 6 and 13 of the study.

Body weight gain and drinking-water consumption were significantly reduced in the mid- and high-dose groups. Blood levels of cyanide and thiocyanate were fairly consistent over time. A dose–response relationship was observed for the concentration of both cyanide and thiocyanate in urine. The ratio of cyanide to thiocyanate in the urine was about 1:1000. A relatively small amount of thiocyanate was observed in the urine of the controls. The study authors reported that after 6 and 13 weeks, 11% of the administered cyanide was eliminated per day as urinary thiocyanate and that only 0.003% was excreted per day unchanged; however, it is unclear how these values were calculated. Radiolabelled material was not used. Some elimination may have occurred as exhaled HCN or carbon dioxide. The study authors also noted that the percentage of administered cyanide excreted via the urine was unchanged between weeks 6 and 13, indicating that detoxification pathways were not saturated and the mode of cyanide excretion was not affected over this period (Leuschner, Winkler & Leuschner, 1991).

(b) Goats

Results of a study administering cyanide by gavage to lactating goats indicate that cyanide and thiocyanate can be transferred through milk to nursing offspring (Soto-Blanco & Górniak, 2003).

In a study of the comparative toxicokinetics of cyanide, 42 male Wistar rats, 6 male Landrace-Large White pigs and 6 male Alpine crossbred goats were given a single oral potassium cyanide dose of 3.0 mg/kg bw (equivalent to 1.2 mg/kg bw expressed as cyanide) by gavage. Blood samples were collected 15 minutes before dosing and at 15 and 30 minutes and 1, 3, 6, 9, 12, 18 and 24 hours after dosing. The peak plasma concentration of cyanide was reached within 30 minutes in pigs and within 15 minutes in rats and goats. Goats had the highest volume of distribution, highest area under the curve and slowest elimination of cyanide compared with the other two species. The peak thiocyanate concentration in plasma was reached within 3 hours in goats and within 6 hours in pigs and rats. Therefore, the study authors concluded that goats would be more sensitive than pigs or rats to the toxic effects of cyanide and thiocyanate (Sousa et al., 2003).

3.1.2 Biotransformation

Cyanide released from the metabolism of the cyanohydrin moiety of a cyanogenic glycoside is readily absorbed and rapidly distributed in the body. Cyanide can be detoxified via first-pass metabolism by conversion to thiocyanate ion, which is considerably less toxic. The major pathway for this conversion is via the intramitochondrial enzyme rhodanese (thiosulfate–cyanide sulfur transferase), a liver enzyme that catalyses the transfer of sulfur from a donor to cyanide to form thiocyanate, which is then readily excreted in urine (Figure 3). Consequently, sulfur-containing donor molecules (divalent bonded sulfur atoms) are the rate-limiting factor in the detoxification of cyanide. Several polymorphisms in rhodanese have been identified in human populations, although only a minimal effect on cyanide detoxification was detected (Billaut-Laden et al., 2006). Although rhodanese is present in the mitochondria of all tissues, the species and tissue distributions of rhodanese are highly variable. It has been reported that the rat has higher rhodanese activity than the dog, Rhesus monkey and rabbit (Himwich & Saunders, 1948). In general, the highest concentrations of rhodanese are found in the liver, kidney, brain and muscle (Aminlari, Vaseghi & Kargar, 1994). Approximately 80% of cyanide is estimated to be detoxified by conversion to thiocyanate, which, once formed, subsequently undergoes renal clearance with a half-life of approximately 2.7 days (Schulz, Bonn & Kindler, 1979; WHO, 2009a), whereas the rate of spontaneous detoxification of cyanide in humans is estimated to be 1 µg/kg bw per minute (Schulz et al., 1982). Cyanide can also be detoxified by direct chemical combination with sulfur-containing amino acids, such as L-cysteine and L-methionine, or by conjugation with hydroxocobalamin to form cyanocobalamin (IPCS, 2004; ATSDR, 2006). Detoxification of cyanide is therefore affected by the presence of nutritional factors, such as sulfur-containing amino acids and vitamin B12.

3.1.3 Effects on enzymes and other biochemical parameters

The Committee previously reviewed the potential of cyanide to inhibit cytochrome oxidase and other metalloenzymes (Annex 1, reference *102*). HCN can inactivate cytochrome oxidase found in the mitochondria of cells by binding to the Fe^{3+}/Fe^{2+} contained in the enzyme. This causes a decrease in the utilization of oxygen in the tissues. The adenosine triphosphatase/adenosine diphosphatase ratio decreases as the cell then shifts to anaerobic metabolism, creating excess lactic acid and a metabolic acidosis. Cyanide activates glycogenolysis and shunts glucose to the pentose phosphate pathway, decreasing the rate of glycolysis and inhibiting the tricarboxylic acid cycle.

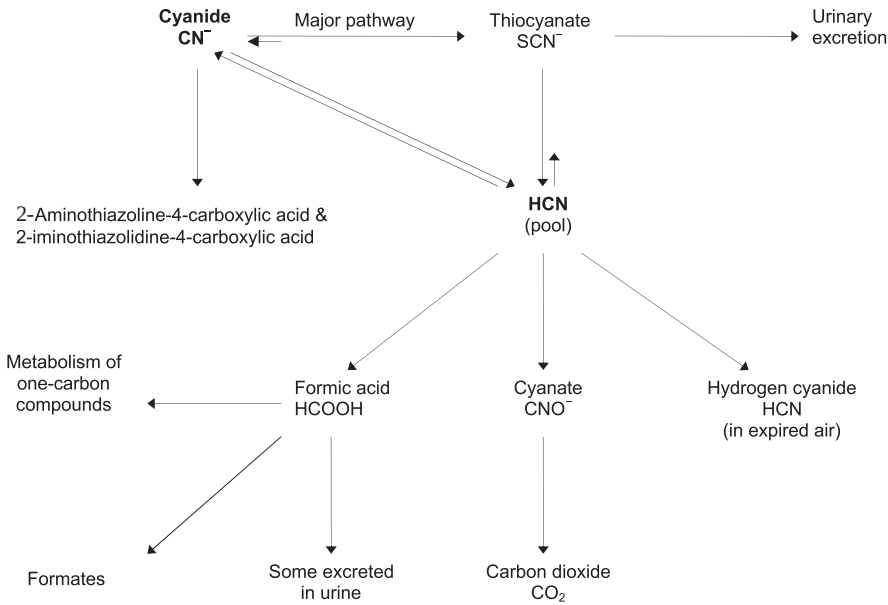
3.2 Toxicological studies

3.2.1 Acute toxicity

The acute toxicity of HCN has been extensively reviewed previously by the Committee, and the following lethal doses of HCN were reported: mouse, 3.7 mg/kg bw; dog, 4.0 mg/kg bw; cat, 2.0 mg/kg bw; and cattle and sheep, 2.0 mg/kg bw.

Oral LD_{50} values for sodium cyanide were calculated as 3 mg/kg bw (as cyanide) for unfasted rats and 2.7 mg/kg bw (as cyanide) for rats that fasted for 24

Figure 3. Primary metabolic pathways of cyanide



Source: Adapted from Ansell & Lewis (1970)

hours before exposure (Ballantyne, 1988). These LD_{50} values are based on bolus doses that exceed the detoxification capacity for cyanide.

3.2.2 Short-term studies of toxicity

(a) Mice

In a study compliant with good laboratory practice (GLP), B6C3F1 mice (10 of each sex per group) were administered sodium cyanide in drinking-water for 13 weeks at concentrations of 0, 3, 10, 30, 100 or 300 mg/l (cyanide doses calculated by study authors as 0, 0.26, 0.96, 2.7, 8.6 and 24.4 mg/kg bw per day, respectively, in male mice and 0, 0.32, 1.1, 3.3, 10.1 and 28.8 mg/kg bw per day, respectively, in female mice). The following parameters were evaluated: body weight, clinical signs, water consumption, clinical chemistry, haematology, urinalysis, extensive histopathology, selected organ weights (heart, kidneys, liver, lungs, thymus gland, testes, epididymis, cauda epididymis), testicular sperm measures (spermatid count, spermatid heads), epididymal sperm measures (spermatozoa count and motility) and vaginal cytology. The cauda subsection of the epididymis functions as a site of sperm maturation and storage. Because the cauda is part of the epididymis, these weights are not independent end-points. Thyroid weight and levels of thyroid hormones were not evaluated.

No significant treatment-related effects on mortality, body weight or clinical end-points were observed. Water consumption in both males and females was decreased (10–30%) in the 100 and 300 mg/l dose groups. Absolute and relative liver weights were significantly increased by 18% and 23%, respectively, in the high-dose females, and relative liver weight was significantly increased in the high-dose males (12%). However, the study authors did not consider the changes in absolute and relative organ weights to be related to sodium cyanide treatment.

No treatment-related effects were observed in clinical chemistry, haematology, urinalysis, non-reproductive organ weights or histopathology in any of the assessed organs. Reproductive effects were evaluated only in mice exposed to the highest three doses (≥2.7 mg/kg bw per day). Statistically significant decreases of 10% and 18% in the absolute weights of the epididymis and cauda epididymis, respectively, were observed in the high-dose group relative to controls. Relative cauda epididymis weight was significantly decreased (18%) in the 8.6 mg/kg bw per day dose group. There were no statistically significant decreases in epididymal and testis weights (relative and absolute) or sperm parameters (spermatozoa per gram cauda epididymis, total spermatozoa per cauda epididymis and spermatozoa motility). No reproductive system effects were reported at any of the dose levels tested for female mice. A NOAEL of 2.7 mg/kg bw per day was determined based on a statistically significant decrease in relative cauda epididymis weight at the next higher dose level (NTP, 1993; USEPA, 2010).

(b) Rats

A 13-week toxicity study with male Sprague-Dawley rats (approximately 30 rats per group) in which potassium cyanide was administered in the drinking-water (40, 80 or 160/140 mg/kg bw per day) was reviewed previously by the Committee. The potassium cyanide in the water decreased its palatability, and this resulted in decreased feed consumption and body weight gain. A NOAEL was not established for this study (Leuschner et al., 1989).

In a GLP-compliant study, F344 rats (10 per group for base study) were administered sodium cyanide in drinking-water for 13 weeks at concentrations of 0, 3, 10, 30, 100 or 300 mg/l (calculated by study authors as cyanide doses of 0, 0.16, 0.48, 1.4, 4.5 and 12.5 mg/kg bw per day, respectively, in male rats and 0, 0.16, 0.53, 1.7, 4.9 and 12.5 mg/kg bw per day, respectively, in female rats). There were additional “clinical pathology” groups of 10 males for blood sampling at days 5, 25 and 45 and week 13. The parameters evaluated included body weight, clinical signs, water consumption, clinical chemistry, haematology, urinalysis, extensive histopathology (control and 300 mg/l groups), selected organ weights (heart, kidneys, liver, lungs, thymus gland, testes, epididymis and cauda epididymis), testicular sperm measures (spermatid count and spermatid heads), epididymal sperm measures (spermatozoa count and motility) and vaginal cytology. Thyroid weight and levels of thyroid hormones were not evaluated in this study.

Treatment-related effects on mortality or clinical signs of toxicity were not seen in either males or females. Compared with the control groups, there was a dose-related decrease in water consumption that was greater than 10% in both sexes exposed to cyanide doses of 4.5 or 12.5 and 4.9 or 12.5 mg/kg bw per

day for males and females, respectively. The study authors attributed the observed decrease in urine volume and increase in urine specific gravity in the high-dose male rats to decreased water consumption.

At the end of the study, urinary thiocyanate concentration was statistically significantly increased at cyanide doses of 1.4 and 1.7 mg/kg bw per day and above in males and females, respectively. A statistically significant increase in absolute (16%) and relative (12%) liver weights in high-dose females relative to controls was observed. There were no observed effects on non-reproductive organ weights in males. No histopathological changes were attributed to cyanide exposure. The durations of proestrus and diestrus were increased in female rats in the 4.9 and 12.5 mg/kg bw per day cyanide dose groups compared with the control group; however, the increase was not considered by the study authors to be treatment related or dose dependent.

Male reproductive end-points in the testis and epididymis were evaluated only in rats exposed to cyanide doses of 1.4 mg/kg bw per day and higher, and only with the left reproductive organ. The weights of the epididymis, cauda epididymis subsection and testis were also measured. Absolute and relative cauda epididymis weights were statistically significantly decreased by cyanide doses of 1.4 mg/kg bw per day and higher compared with the control group; however, only the decrease in absolute weights was considered dose dependent. A statistically significant decrease in epididymal sperm motility was observed at cyanide doses of 1.4 mg/kg bw per day and higher. Testicular spermatid count was statistically significantly decreased in the highest cyanide dose group (12.5 mg/kg bw per day), although epididymal sperm count was not affected at any dose tested. No morphological effects were observed in male reproductive tissues examined using standard histopathological techniques (NTP, 1993; USEPA, 2010).

Sixty-three female Wistar rats were divided into seven equal groups and treated by gavage daily for 14 days. The treatment groups were as follows: 1) control, 2) α-ketoglutarate, 1.0 g/kg bw per day, 3) sodium thiosulfate, 1.0 g/kg bw per day, 4) potassium cyanide, 7.0 mg/kg bw per day, 5) potassium cyanide + α-ketoglutarate, 6) potassium cyanide + sodium thiosulfate and 7) potassium cyanide + α-ketoglutarate + sodium thiosulfate. The sodium thiosulfate was given intraperitoneally. Various haematological and biochemical indices were determined after 7 days of treatment; after 14 and 21 days (recovery group) of cyanide exposure, additional parameters, such as organ–body weight index, were measured, and histology of brain, heart, lung, liver, kidney and spleen was performed.

The 14-day exposure to potassium cyanide at 7 mg/kg bw per day did not produce any significant change in body weight of the animals, organ–body weight index, haematology or the levels of blood urea, creatinine, aspartate aminotransferase, triiodothyronine (T_3) and thyroxine (T_4). The levels of temporal glutathione disulfide and hepatic malondialdehyde, reduced glutathione (GSH) and glutathione disulfide were unaffected. However, in potassium cyanide–treated animals, elevated levels of blood glucose and reduced levels of alanine aminotransferase (ALT) were observed. Activities of cytochrome *c* oxidase in the brain and rhodanese in the liver were diminished. Reduced levels of GSH and enhanced levels of malondialdehyde in brain were observed. Increased levels of

blood thiocyanate were observed in all animals treated with potassium cyanide. Potassium cyanide also produced various histological changes in the brain, heart, liver and kidney (Tulsawani et al., 2005).

(c) Rabbits

Groups of six male rabbits were fed a diet of growers' mash only or mash containing cyanide (as potassium cyanide) at a concentration of 702 mg/kg (cyanide doses calculated as 0.2 and 20 mg/kg bw per day, respectively) for 10 months. Decreases of 33% for both body weight and feed efficiency were reported in the high-dose group. Serum concentrations of ALT, alkaline phosphatase, lactate dehydrogenase and sorbitol dehydrogenase were increased in treated rabbits by the end of the treatment period. Alkaline phosphatase concentrations were reduced in the lung but not in the heart. The study authors considered the increase in lactate dehydrogenase activity in the liver and kidney as evidence of a shift from aerobic to anaerobic metabolism. Histopathological findings of focal areas of hepatic necrosis and congestion in addition to renal tubular and glomerular necrosis supported the biochemical evidence of tissue injury in the liver and kidney. Focal pulmonary oedema and necrosis in treated rabbits were also reported. A NOAEL was not established (Okolie & Osagie, 1999, 2000).

3.2.3 Long-term studies of toxicity and carcinogenicity

The Committee previously reviewed a study in which the longevity of mice with transplanted Ehrlich ascites tumours and Sarcoma 180 was increased 20–70% on intraperitoneal injection of sodium cyanide in the dose range 0.75–2.0 mg/kg bw (USEPA, 1990).

3.2.4 Genotoxicity

The Committee previously reviewed a variety of studies, including those with potassium cyanide or HCN and *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537 and TA1538, with or without S9 liver microsomes, a study in which Chinese hamsters were orally administered HCN for the detection of chromosomal aberrations and a study with potassium cyanide and cultured Chinese hamster cells, V79 (genetic marker HGPRT), in both the presence and absence of a metabolic activation system. The results of these studies did not provide evidence that HCN or potassium cyanide exhibits genotoxic activity.

No new studies have been identified since the last evaluation by the Committee.

3.2.5 Reproductive and developmental toxicity

(a) Rats

A short-term reproductive study (49-day study in adults and 28-day study in pups) to evaluate the cumulative effects of adding potassium cyanide (500 mg/kg) to cassava root flour–based diet in pregnant rats was previously reviewed by this

Committee (Annex 1, reference *102*). This meal was prepared from a low-cyanide cassava variety (cyanide concentration of 21 mg/kg feed) and supplemented with other dietary components to give a final diet containing HCN at 12 mg/kg diet. Gestation and lactation performances were not affected. However, the high-cyanide diet significantly reduced feed consumption and daily growth rate of the offspring when fed during the postweaning period. Serum thiocyanate levels were significantly increased in lactating rats and their offspring during lactation and in the postweaning growth phase of the pups. Rhodanese activity in liver and kidneys was unaffected by feeding the high-cyanide diet during gestation, lactation and postweaning growth (Tewe & Maner, 1981).

Twenty pregnant female Wistar rats were fed potassium cyanide at 0 or 500 mg/kg (equivalent to a cyanide dose of 0 or 20 mg/kg bw per day) in the diet during gestation and up to postnatal day (PND) 50. Offspring (five per group) were sacrificed on PNDs 1, 9, 14, 21, 28 and 50; parameters examined included body weight, brain weight, cerebellar weight, maximum vermis length (length between cerebellar hemispheres), maximum side-to-side dimensions of the cerebellum and maximum thickness (anteroposterior dimension) of the cerebellum. Aggressive and restless behaviour was noted in the exposed dams but not in controls. Significantly decreased body weight (6%) and brain weight (19%) were observed in the treated pups on PNDs 14 and 9, respectively. No significant changes in body weight or brain weight were found at the other time points examined. Cerebellar weight was significantly reduced on PNDs 14, 21 and 28. The maximum vermal length was significantly reduced on day 50, and the maximum side-to-side width of the cerebellum was reduced on day 28 (Imosemi et al., 2005).

Microscopic parameters of the cerebellum were reported in a separate publication by Malomo et al. (2004). A significantly thicker external granular layer was seen in the experimental group on PNDs 14 and 21, which was suggested by the study authors to indicate delayed maturation and migration of cells in the cerebellum. Reduced thickness of the molecular layer was also observed on PNDs 28 and 50. The density and size of the Purkinje cells were not different between groups. There was evidence of normal myelination based on staining of the white matter that was similar between groups. The study authors concluded that maternal consumption of cyanide at 20 mg/kg bw per day did not significantly affect microscopic indicators of cerebellar development, but caused mild changes later in postnatal life. A LOAEL of 20 mg/kg bw per day for cyanide was identified based on altered cerebellar development (Malomo et al., 2004).

(b) Hamsters

The Committee (Annex 1, reference *102*) previously reviewed a study in which pregnant Golden hamsters were exposed to sodium cyanide on days 6–9 of gestation by infusion via subcutaneously implanted osmotic minipumps. Cyanide (0.126–0.1295 mmol/kg bw per hour, approximately equal to 80 mg/kg bw per day) induced high incidences of resorptions and malformations in the offspring. The most common abnormalities observed were neural tube defects (Doherty, Ferm & Smith, 1982).

(c) Goats

The effects of gestational exposure to cyanide by the oral route in pregnant mixed-breed goats were evaluated. Goats were administered two daily gavage doses of potassium cyanide totalling 0, 1, 2 or 3 mg/kg bw per day (equivalent to cyanide doses of 0, 0.4, 0.8 or 1.2 mg/kg bw per day) from day 24 (day 1, post-implantation) of pregnancy until parturition (day 150). There were eight goats in the control and high-dose groups and five in the other two groups. Body weights were determined weekly. Blood samples were collected every other week and analysed for plasma glucose, cholesterol and thiocyanate levels. At birth, body weight and sex were recorded, and each neonate was examined for gross abnormalities. Body weights and body weight gain were recorded weekly from the day of birth for 3 months. T_4 and T_3 concentrations in plasma from the offspring were measured at birth (day 1) and on day 7, and glucose and cholesterol concentrations were measured on days 1, 7, 45 and 90. One control dam and one dam from the highest dose group were sacrificed on day 120 for histopathological assessment. Three months after birth, the male offspring and one dam from each group were sacrificed, and the pancreas, thyroid and entire central nervous system (including spinal cord) were collected for histological examination.

During treatment, two dams from the highest dose group experienced clinical signs of cyanide intoxication, specifically ataxia and convulsions, and were treated intravenously with sodium nitrate and sodium thiosulfate. Cyanide treatment did not significantly alter body weight gain or length of gestation. Two prognata kids were born from different dams in the high-dose group. In addition, another dam in the high-dose group aborted two fetuses, one of which was prognata. T_3 levels in dams and offspring were significantly elevated over controls in the highest dose group on day 1 but not day 8; T_4 levels were not significantly different compared with controls. The dam sacrificed on day 120 of pregnancy demonstrated increased reabsorption vacuoles in the thyroid follicular colloid and severe spongiosis of the cerebral, internal capsule and cerebellar peduncle white tracts, suggestive of myelin oedema of the white matter, but no morphological alterations of the pancreas. No lesions were reported in the histopathological examination of dams and offspring 3 months after birth. A LOAEL cannot be identified for this study, because incidence and severity data of the observed histological effects were not reported (Soto-Blanco & Górniak, 2004).

3.2.6 Special studies on the thyroid gland

(a) Rats

The Committee (Annex 1, reference *102*) previously reviewed a study in which male rats receiving a diet containing potassium cyanide at 0 or 1500 mg/kg feed for nearly 1 year exhibited decreased plasma T_4 levels and T_4 secretion rates after 4 months, but less so after 1 year. No lesions in the thyroid gland were observed by light microscopy (Philbrick et al., 1979).

(b) Dogs

The toxicity of inorganic cyanide administered in a rice diet to male dogs (six per group) for 14 weeks was evaluated. The diet was supplemented at feeding

time with sodium cyanide to provide a cyanide dose of 1.04 mg/kg bw per day. The dogs were of mixed breed and purchased from local African markets at 6 weeks of age; treatment was initiated when the dogs were approximately 22 weeks old. The dogs were repeatedly treated for ectoparasites and endoparasites. It is unclear what impact the compromised health status and repeated treatment for parasites had on the observed effects in the dogs. In the testes, specialized reproductive morphological analysis indicated that the treated dogs had a significantly decreased percentage of tubules in stage VIII of the spermatogenic cycle (characterized by elongated spermatids lining the lumen of the seminiferous tubules) compared with controls ($P < 0.01$). This percentage was 1.6% ± 1.07% (mean ± standard error of the mean [SEM]) in the treated group compared with 14.4% ± 0.94% in the controls. Treated dogs also had an increased incidence of abnormal cells and sloughing of germ cells in the seminiferous tubules. Hyperplasia and hypertrophy were observed in the adrenal gland. The adrenal medulla was unaffected by inorganic cyanide treatment. Although the width of the adrenal cortex did not differ significantly between the cyanide-treated and control groups, the zona glomerulosa (the most superficial layer of the adrenal cortex) was significantly wider in treated dogs. The results of this study indicate that cyanide may be a reproductive toxicant in male dogs (Kamalu, 1993).

An evaluation of the thyroid from this study at week 14 showed that the serum T_3 level was significantly decreased by 55% and thyroid weight was significantly increased by 23% in the cyanide-exposed group (compared with control animals at the same time point). A histopathological evaluation of the thyroid gland found decreased colloid content compared with that of controls (Kamalu & Agharanya, 1991).

(c) Pigs

The Committee (Annex 1, reference *102*) previously reviewed a study assessing metabolic and pathological changes in 48 growing pigs fed different levels of dietary protein (9% and 16%), cyanide and iodine (0 and 0.36 mg/kg feed) over 56 days. The histopathology of the examined tissues did not indicate any treatment-related effects. Dietary iodine deficiency caused histological changes in the thyroid gland (hyperplastic goitre) and bone, which suggested a decline in metabolic activity (Tewe & Maner, 1980).

For 24 weeks, daily doses of potassium cyanide, equivalent to cyanide doses of 0, 0.4, 0.7 or 1.2 mg/kg bw per day, were administered to three mini-pigs per group via gavage prior to feeding. Regularly measured serum thiocyanate levels were positively correlated with cyanide dose. Serum levels of T_3, T_4 and glucose were measured every 6 weeks. Performance measures, including innate behaviour, and learning measures, including the acquisition and retention of new behaviours, were evaluated daily.

By week 18, T_3 and T_4 levels were statistically significantly decreased in the high-dose group, by 23% and 13%, respectively. The overall pattern of behavioural changes in the high-dose group was reported to be different from that of the control animals, but the changes at lower doses were inconsistent. Based on reported behavioural changes and decreased thyroid hormones, the LOAEL and NOAEL

values identified for this study are 1.2 and 0.7 mg/kg bw per day, as cyanide, respectively (Jackson, 1988).

The effects of subchronic (70-day) potassium cyanide ingestion were examined in 45-day-old Lanrace-Large White pigs. The number and sex of animals were inconsistently reported in this publication; however, dose groups, based on the summary data tables, ranged between 5 and 10 pigs per group. Twice each day, the pigs were given potassium cyanide mixed with 10 g starch biscuits, for total daily doses of 0, 2, 4 or 6 mg/kg bw per day (cyanide doses of 0, 0.8, 1.6 and 2.4 mg/kg bw per day). Blood samples were collected prior to the experimental period and then every week thereafter and analysed for ALT, glucose, cholesterol, blood urea nitrogen, creatinine, T_3, T_4 and thiocyanate. At terminal necropsy, thyroid glands were weighed, and tissues from the central nervous system, thyroid, pancreas, liver and kidneys were examined histologically. Significantly decreased serum ALT was observed at and above 0.8 mg/kg bw per day. Additionally, significantly increased urea and creatinine levels were observed at and above 1.6 mg/kg bw per day. Thyroid weight increased by 24% in the highest dose group compared with the control group, although significant alterations in thyroid hormones were not observed. Histopathology of the thyroid gland found numerous vacuoles in the colloid of the thyroid follicles in all dose groups. Liver lesions were reported as karyolysis, pyknosis and distortion of the normal lobular architecture. Renal tubular epithelial degeneration was reported in the kidney. In the brain, minimal degeneration of Purkinje cells and loss of cerebellar white matter were reported. The pancreas was not affected by treatment. All histological lesions were reported by the authors to occur in a dose-related manner, although neither incidence nor statistical analysis of these findings was presented. A LOAEL of 2.4 mg/kg bw per day based on increased thyroid weight was determined for this study (Manzano et al., 2007).

3.2.7 Special studies on the nervous system

The Committee (Annex 1, reference *102*) previously reviewed neuronal lesions from several studies and species produced by chronic cyanide intoxication either by injection of unbuffered alkaline cyanide salts or by inhalation of HCN. Reported neuropathological changes included areas of focal necrosis especially around the centrum ovale, corpus striatum, corpus callosum, substantia nigra and anterior horn cells and patchy demyelination in the periventricular region.

(a) Rats

Wistar rats (10 per group) were given potassium cyanide by oral gavage at daily doses of 1.4 mg/kg bw per day or distilled water for 90 consecutive days. Body weight was recorded (not reported), brain, liver and kidney were weighed and histopathological examination of these organs was performed. Spontaneous motor activity and motor coordination were recorded every 15th day. Lipid peroxidase, GSH, glutathione peroxidase, superoxide dismutase and catalase activities in blood, brain, liver and kidney and glutamate, aspartate and dopamine levels in discrete regions of brain were measured after 90 days of treatment.

Lesions of histopathological significance were not observed in the brain or kidney of the treated rats, whereas microgranuloma, spotty necrosis and moderate portal inflammation were observed in the liver. Compared with the control group, potassium cyanide exposure significantly decreased motor coordination, accompanied by an increase in lipid peroxidation (blood, brain and liver) and dopamine concentration (corpus striatum and cerebral cortex) and depletion of GSH (blood, brain and liver), glutathione peroxidase (brain and liver), superoxide dismutase (brain and liver) and catalase (blood and brain) concentrations. The study authors concluded that oral exposure to cyanide induced oxidative stress in the brain and liver (Mathangi et al., 2011).

Groups of male Wistar rats were administered potassium cyanide daily at doses of 0, 0.15, 0.3 or 0.6 mg/kg bw per day, equivalent to cyanide doses of 0, 0.06, 0.12 and 0.24 mg/kg bw per day, by gavage for 12 weeks. The number of animals included in each group was seven, six, six and seven for the control, low-dose, mid-dose and high-dose groups, respectively. The parameters evaluated included clinical signs of toxicity, body weight, feed consumption, serum cholesterol, glucose, T_3 and T_4 levels and histopathology (central nervous system, thyroid, pancreas).

No treatment-related effects were reported for clinical signs of toxicity, body weight changes, feed consumption, serum T_3 and T_4 or serum glucose concentrations. Reported histopathological changes included a dose-related increase in lesions of the spinal cord (spheroid bodies on white matter), neuronal loss in the hippocampus, damaged Purkinje cells and loss of white matter in the cerebellum (Soto-Blanco, Maiorka & Górniak, 2002a).

Forty male albino Wistar strain rats, half of which were maintained on a nutrient-deficient diet and were considered to be malnourished, were divided into four equal groups and administered a daily intraperitoneal injection for 30 days. The control and malnourished control groups received saline, and the treatment and malnourished treatment groups received sodium cyanide at a dose of 2 mg/kg bw. To evaluate memory, rats were administered weekly T-maze tests (10 trials per rat). After sacrifice, the hippocampus was dissected, weighed and retained for neurotransmitter analysis. The authors reported no difference in body weight change for the cyanide-treated rats on both diets when compared with their respective control group. Reductions in memory and concentrations of dopamine and 5-hydroxytryptamine and increases in norepinephrine and epinephrine levels in the hippocampus were observed in the cyanide-treated groups compared with controls. The differences in neurotransmitter concentrations between the two cyanide-treated groups were statistically significantly different (Mathangi & Namasivayam, 2000a).

(b) Pigs

A special study on the behavioural effects of oral potassium cyanide exposure (cyanide doses of 0.4, 0.7 and 1.2 mg/kg bw per day) over 24 weeks in miniature pigs was previously reviewed by the Committee (Annex 1, reference *102*). Slower response times to various stimuli, reduced exploratory behaviour, elevated fasting blood glucose values and dose-related decreases in T_3 and T_4 levels were reported in treated animals (Jackson, 1988).

(c) Goats

Groups of male Alpine-Saanen goats were administered potassium cyanide daily in milk or water for 5 months at doses of 0, 0.3, 0.6, 1.2 or 3.0 mg/kg bw per day (equivalent to cyanide doses of 0, 0.12, 0.24, 0.48 and 1.2 mg/kg bw per day). Potassium cyanide was administered twice daily in milk (one half of the daily dose per treatment) for the first 3 months and in water for the last 2 months. The number of animals included in each group was six, seven, six, seven and eight for the control, low-dose, low mid-dose, high mid-dose and high-dose groups, respectively. The central nervous system of each goat was collected for histopathological examination and immunohistochemistry for apoptotic markers (BAX, BCl2 and CPP32) and for glial fibrillary acid protein (GFAP) and vimentin.

The only clinical signs of toxicity reported were generalized muscular tremors and ataxia in one goat in the high-dose group occurring after potassium cyanide administration on days 121–123, which lasted approximately 30 seconds. Neuropathological lesions observed in the two highest dose groups included congestion, haemorrhage and gliosis of the cerebellum, spinal cord and pons, and spheroids on the grey matter of the spinal cord. Damage and loss of Purkinje cells in the cerebellum, spongiosis in the pons, and spheroids, axonal swelling, gliosis, spongiosis and ghost cells in the medulla oblongata were observed in the high-dose group. The immunohistochemical analysis did not detect the tested apoptotic markers (Soto-Blanco, Maiorka & Górniak, 2002b).

In a 30-day study, 16 Boer-Spanish crossbred female goats were divided into four equal groups that received water and hay or a gavage dose of potassium cyanide, potassium thiocyanate or ground frozen chokecherry leaves and flowers at a targeted HCN equivalent dose of 2.5 mg/kg bw per day. The administration of all substances was divided into two daily doses. The parameters evaluated included body weight, clinical signs, feed consumption, haematology, clinical chemistry and histopathology (pancreas, thyroid gland, liver, kidney, lung, heart, skeletal muscle, spleen, rumen, intestine, sciatic nerve, whole central nervous system). Morphometric evaluation of thyroid follicles (500 per animal) was performed.

No significant treatment-related effects were observed in body weight changes, feed intake, organ weights, haematology or clinical chemistry (including T_4). One chokecherry-treated goat exhibited convulsions and trembling by day 6; subsequently, the dosage was reduced by 25%. The only tissues reported with histological lesions were the thyroid (statistically significant increased number of vacuoles in the chokecherry-treated group) and nervous tissues (evidence of spongiosis and spheroids in the mesencephalon of goats treated with potassium cyanide and chokecherry) (Soto-Blanco et al., 2008).

3.3 Observations in humans

3.3.1 Biomarkers of exposure

Cyanide is concentrated in erythrocytes, and a relatively small proportion is transported via plasma. Low levels of cyanide are found in normal blood of humans without known occupational cyanide exposure, mostly related to tobacco

smoke, cyanogenic food and vitamin B12. The mean levels of cyanide found in non-smoking adults (Lundquist, Rosling & Sörbo, 1985) were 0.13, 0.24, and 0.02 µmol/l in whole blood, erythrocytes and plasma, respectively. Using the 97.5th percentile (mean + two standard deviations [SD]) as a reference level, the resulting value for the concentration in blood is 0.3 µmol/l (3.4 µg/l, or 0.0034 mg/l). Although it seems that levels in plasma reflect tissue levels better than those in whole blood, the latter are often measured as an indicator of exposure in cyanide poisoning. The amount of cyanide in the blood likely to cause toxicity is imprecise and depends on the time between exposure and sample drawing, the specific cyanogenic compound involved, the route of exposure, treatment provided before sampling (if any) and sample handling before analysis. Rough estimates of blood cyanide levels and associated effects in adults (Linden & Lovejoy, 1998) are as follows: flushing and tachycardia at 0.5–1 mg/l, obtundation (dulled sensibility) at 1–2.5 mg/l, coma and respiratory depression at 2.5–3.0 mg/l and death at blood cyanide levels greater than 3.0 mg/l.

3.3.2 Acute toxicity

The acute oral toxicity of HCN in humans, which has previously been reviewed by the Committee (Annex 1, reference *102*), is discussed in section 2.3.2(a) above, as the acute toxicity associated with exposure to cyanogenic glycosides is due to the formation of cyanide.

3.3.3 Epidemiological studies

No subchronic or chronic dose–response studies of human exposure to cyanide by the oral route could be found.

4. ANALYTICAL METHODS

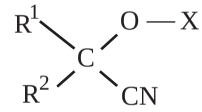
4.1 Chemistry

Table 4 shows the chemical structures of several commonly occurring cyanogenic glycosides and some of the plants in which they are found (FAO/WHO, 2008b).

The toxicity of cyanogenic glycosides is due to their hydrolytic breakdown to sugars and cyanohydrins that, under neutral or mildly alkaline conditions, spontaneously release HCN (see Figure 2 above). The cyanohydrins have been shown to be relatively stable at lower pH values, and this has particular relevance for effective food processing and dietary exposure to HCN (see below) (Haque & Bradbury, 2002; Bradbury, 2009).

The cyanogenic glycosides and their corresponding hydrolytic enzymes (β-glycosidases) are physically separated in plant tissues and are brought together when the cell structure of a cyanogenic plant is disrupted, with subsequent release of HCN.

Table 4. General structure of common cyanogenic glycosides



Name	Formula Molecular mass CAS No.	R^1	R^2	X	Configuration	Occurrence[a]
Amygdalin	$C_{20}H_{27}NO_{11}$ 457.4334 29883-15-6	Phenyl	H	Gentiobiose	R	Almonds, peach, apricot, apple and quince kernels
Linamarin	$C_{10}H_{17}NO_6$ 247.2474 554-35-8	Methyl	Methyl	Glucose	—	Cassava, lima bean, (flaxseed)
Prunasin	$C_{14}H_{17}NO_6$ 295.29 99-18-3	Phenyl	H	Glucose	R	Ferns, e.g. bracken fern, rowanberries
Linustatin	$C_{16}H_{27}NO_{11}$ 409.39 72229-40-4	Methyl	Methyl	Gentiobiose	—	Flax seed, cassava
Lotaustralin	$C_{11}H_{19}NO_6$ 261.272 534-67-8	Methyl	Ethyl	Glucose	R	Lima bean, (cassava), (flaxseed)

Name	Formula Molecular mass CAS No.	R^1	R^2	X	Configuration	Occurrence[a]
Neolinustatin	$C_{17}H_{29}NO_{11}$ 423.42 72229-42-6	Methyl	Ethyl	Gentiobiose	R	Flaxseed
Sambunigrin	$C_{14}H_{17}NO_6$ 295.29 138-53-4	Phenyl	H	Glucose	S	Elderberries
Taxiphyllin	$C_{14}H_{17}NO_7$ 311.29 21401-21-8	*p*-Hydroxyphenyl	H	Glucose	R	Bamboo shoot
Dhurrin	$C_{14}H_{17}NO_7$ 311.29 499-20-7	*p*-Hydroxyphenyl	H	Glucose	S	Durra, (sorghum)

CAS, Chemical Abstracts Service

[a] Minor sources are indicated in parentheses.

Source: FAO/WHO (2008b)

4.2 Description of analytical methods

4.2.1 Introduction

The compounds of health concern found in food derived from cyanogenic plants are 1) the cyanogenic glycosides present in plants used as food, 2) the cyanogenic glycosides and cyanohydrins that remain in food after processing and food preparation and, ultimately, 3) the HCN/CN^- that is present in food or that could be released from cyanogenic glycosides and cyanohydrins remaining in food when it is consumed.

Either cyanogenic glycosides are quantified individually (see section 4.2.3(a)) or the total HCN content of the cyanogenic glycosides and cyanohydrins present in a food is measured (see section 4.2.3(b)). In the latter case, complete hydrolytic formation of cyanohydrins from all of the cyanogenic glycosides present in a particular plant or item of food is followed by measurement of the HCN subsequently released (see Figure 2 above) (EFSA, 2007a; FAO/WHO, 2008b). The term "total HCN" was used by the Committee to describe the total HCN content of all cyanogenic glycosides, cyanohydrins and "free" HCN in a food.

Codex maximum levels (MLs) for cyanogens are expressed as milligrams of total HCN per kilogram of food (e.g. Codex Standard 176-1989), so that with analytical methods measuring cyanogenic glycoside concentrations, the levels found must be converted stoichiometrically to total (potential) HCN concentrations to determine if they conform to the MLs. For example, 1 g of amygdalin, linamarin and prunasin shown in Table 4 each liberate the following quantities of HCN: amygdalin, 59.1 mg HCN; linamarin, 109.3 mg HCN; and prunasin, 91.5 mg HCN (FAO/WHO, 2008b).

4.2.2 Screening tests

The most common screening methods for cyanogenic glycosides present in plants used for food are colorimetric tests for HCN released following disruption of plant tissues. Many such tests have been described and have been used to identify thousands of cyanogenic plant species (Feigl & Anger, 1966; Eyjolfsson, 1970; Cooke, 1978; Bradbury, Egan & Lynch, 1992; Vetter, 2000), including those used as food (Jones, 1997). One of the earliest and perhaps currently the most commonly employed screening test is predicated on the brick-red colour generated when HCN reacts with sodium picrate adsorbed on paper strips. It has been developed as a generally applicable quantitative test for the total HCN potential of a wide range of unprocessed food (Haque & Bradbury, 2002) and is described in greater detail in a later section (section 4.2.3(b)).

4.2.3 Quantitative methods

(a) Quantification of individual cyanogenic glycosides

To quantify individual cyanogenic glycosides in plants and foodstuffs derived from them, various methods have been employed, including high-performance liquid chromatography (HPLC), gas–liquid chromatography (GLC), TLC, enzyme immunoassay and micellar capillary electrophoresis.

Methods of extracting cyanogenic glycosides from various matrices for identification and analysis include using aqueous alcohol mixtures at different temperatures, extraction times and methods of agitation (Bacala & Barthet, 2007). Extraction methods for flaxseed were recently compared (Bacala & Barthet, 2007), and significant differences were found to result from choice of grinder and extraction method. It was concluded that extraction efficiency would vary considerably across all of the published extraction methodologies, and this could, in some cases, lead to significant underestimation of the amount of cyanogenic glycosides and cyanide potential (Barthet & Bacala, 2010).

HPLC has been widely used to isolate, identify and quantify individual cyanogenic glycosides (Cairns et al., 1978; Schilcher & Wilkens-Sauer, 1986; Frehner, Scalet & Conn, 1990; Oomah, Mazza & Naschuk, 1992; Swain, Li & Poulton, 1992; Amarowicz, Chong & Shahidi, 1993; Cunnane et al., 1993; Wanasundara et al., 1993; Stochmal & Oleszek, 1994; Kolodziejczyk & Fedec, 1995; Kobaisy, Oomah & Mazza, 1996; Krech & Fieldes, 2003; Park et al., 2005). Kobaisy, Oomah & Mazza (1996) compared an HPLC method for cyanogenic glycosides with two enzyme-based colorimetric methods for total HCN and found no statistical difference, although the total HCN levels obtained stoichiometrically from cyanogenic glycoside concentrations were slightly higher than those given by the colorimetric methods: a mean of 70.9 mg/100 g for HPLC compared with means between 59.4 and 62.6 mg/100 g for the two colorimetric methods.

GLC has also been used to separate and quantify individual cyanogenic glycosides as their trimethylsilyl or trifluoroacetyl derivatives (Bissett et al., 1969; Nahrstedt, 1970, 1973; Seigler, 1975; Cairns et al., 1978; Frehner, Scalet & Conn, 1990; Chassagne et al., 1996; Bacala & Barthet, 2007). By using a mass spectrometer as a GLC (or HPLC) detector, additional molecular characterization data and confirmation of identity are obtained (Cairns et al., 1978). Bacala & Barthet (2007) developed a comprehensive extraction and GLC method for measuring linustatin and neolinustatin in flaxseed. The method is capable of processing up to 90 samples per day and has a limit of detection (LOD) for linustatin and neolinustatin of between 0.212 and 2.06 µg/ml (lower limit of quantification [LOQ], 40 µg/ml).

TLC has also been used to analyse individual cyanogenic glycosides (Palmer et al., 1980; Smith et al., 1980; Brimer et al., 1983; Selmar, Lieberei & Biehl, 1988; Amarowicz, Wanasundara & Shahidi, 1993a; Niedźwiedź-Siegieň, 1998; Lautie, Stankovic & Sinoquet, 2000; Haque & Bradbury, 2002; Kuti & Konoru, 2006), but it is generally considered to be a semiquantitative method.

Cho et al. (2006) raised antisera in rabbits reactive to amygdalin using an amygdalin-keyhole limpet haemocyanin conjugate, and the polyclonal antisera have been used to produce a competitive enzyme immunoassay (enzyme-linked immunosorbent assay [ELISA]) for this cyanogenic glycoside, with an LOD of 2.7 $\times 10^{-7}$ mmol/l, which compares favourably with that of other assays. Recombinant antibodies against amygdalin have been used to produce a more sensitive competitive ELISA for amygdalin, with an LOD of 1×10^{-9} mol/l (Cho et al., 2008). ELISA methods offer several benefits, including ease of automation and low cost per assay, and are likely to have a future in analysing individual cyanogenic glycosides in foodstuffs.

Micellar capillary electrophoresis has been used to rapidly separate and quantify amygdalin, prunasin and their isomers neoamygdalin and sambunigrin, with an LOD of 5 µmol/l (Campa et al., 2000).

(b) Quantification of total cyanide potential

Hydrolytic release of total HCN from cyanogenic glycosides in plant and/or food samples (see Figure 2 above) has been achieved by spontaneous, endogenous enzymatic hydrolysis in macerated plant material (Butler, Bailey & Kennedy, 1965; Fan & Conn, 1985; Chadha, Lawrence & Ratnayake, 1995) or, preferably, adding the appropriate β-glucosidase enzymes to ensure complete conversion of cyanogenic glycosides to cyanohydrins, which then decompose spontaneously or with enzymatic assistance to HCN (see Figure 2 above) (Cooke, 1978; O'Brien, Taylor & Poulter, 1991; Essers et al., 1993; Essers, 1994; Haque & Bradbury, 2002).

A difficulty associated with relying on endogenous glycosylase activity to release HCN is that some cultivars are deficient in or lack these enzymes, and the enzymes are also destroyed by processing. A shortcoming in using added enzymes for liberating cyanide is that specific glucosidases may be required for different cyanogenic glycosides, and in some cases (e.g. flaxseed containing linstatin and neolinstatin), two specific enzymes are required: one, linstatinase, converts them to linamarin and lotaustralin, respectively, and the other, linamarase, liberates cyanide (Bacala & Barthet, 2007).

An alternative method for determining total HCN, which has the advantage of overcoming enzyme deficiencies and is therefore widely applicable to both fresh and processed foods, is to use dilute acid (e.g. 2 or 4 mol/l sulfuric acid) with heating at 100 °C, rather than relying on enzymes, to hydrolyse the cyanogenic glycosides present in samples to the corresponding cyanohydrins, which, on basification, release HCN/CN^- (Lambert, Ramasamy & Paukstelis, 1975; Nagashima, 1978; Mendoza et al., 1984; Bradbury, Egan & Lynch, 1991; Essers et al., 1993; Bradbury, Bradbury & Egan, 1994; Haque & Bradbury, 2002; ESR, 2010).

Methods of quantifying the released HCN include colorimetry, GLC, HPLC and biosensors.

To ensure that all HCN released from cyanogenic glycosides is retained for analysis, macerated or ground plant material or food samples, with or without added enzymes or dilute acid, are incubated in sealed containers. In one commonly used enzyme-based colorimetric method, the liberated HCN is allowed to react directly with sodium picrate–impregnated paper strips suspended above the solution to give a brick-red response or, less commonly, with paper strips with guaiacum resin treated with dilute copper sulfate to give a blue response that can be assessed visually against colour standards or, more accurately, using a spectrophotometer after extracting the chromogen from the paper strips (Lambert, Ramasamy & Paukstelis, 1975; Seigler, 1991; Brinker & Seigler, 1992; Essers et al., 1993; Bradbury, Bradbury & Egan, 1994; Yeoh & Egan, 1997; Alonso-Amelot & Oliveros, 2000; Yeoh & Sun, 2001; Haque & Bradbury, 2002; Bradbury, 2009).

As an alternative to the enzyme–picrate method, three other colorimetric reagents, based on the König reaction (König, 1904), have been employed to

measure total HCN following hydrolysis (Lambert, Ramasamy & Paukstelis, 1975; Nagashima, 1978; Mendoza et al., 1984; Bradbury, Egan & Lynch, 1991; Essers et al., 1993; Bradbury, Bradbury & Egan, 1994; Haque & Bradbury, 2002). When hydrolysis is complete, the reaction mixture is basified with sodium hydroxide, and an aliquot in pH 5.0 buffer is treated with chloramine-T to convert the sodium cyanide to cyanogen chloride, followed by the addition of pyridine/barbituric acid or pyridine/pyrazolone/bispyrazolone or isonicotinic acid/barbituric acid to generate the chromogen (Bradbury, Bradbury & Egan, 1994). Diethyl barbituric acid can also be used in place of barbituric acid. Bradbury, Bradbury & Egan (1994) compared these three reagents, optimized their application for HCN analysis and concluded that use of the isonicotinic acid/barbituric acid reagent, with spectrophotometer readings at 600 nm, taken at an absorbance plateau between pH 5.5 and pH 6.5, was the preferred method.

Haque & Bradbury (2002) later generalized both the enzyme–picrate and acid hydrolysis–König methods to enable the total HCN content of any food or plant sample to be determined with a limit of sensitivity of 5 mg/kg using a colour chart or 1 mg/kg using spectrophotometry (Bradbury, 2009). A recent, more sensitive enzyme–picrate method to determine total cyanide (mg HCN equivalents) in gari from cassava is applicable in the HCN range 0.1–50 mg/kg (Bradbury, 2009). Haque & Bradbury (2002) also compared an acid hydrolysis colorimetric method involving chloramine-T and isonicotinic acid/barbituric acid with the enzyme–picrate colorimetric method and found that the acid hydrolysis method, although generally applicable to all plants and foodstuffs, was more difficult, less reproducible and less accurate than the enzyme–picrate method in regard to cassava analysis. In a recent, comprehensive survey of total HCN in a range of plant-based foodstuffs conducted in New Zealand (ESR, 2010), the acid hydrolysis–isonicotinic acid/barbituric acid method of Haque & Bradbury (2002) was the method selected for total HCN analysis on the basis of its general applicability to a variety of processed foods containing structurally diverse cyanogenic glycosides (ESR, 2010). The LOD reported was 4 mg of HCN per kilogram for single analyses and 3 mg of HCN per kilogram for duplicate analyses (ESR, 2010). One quarter of the samples were analysed in duplicate, and the overall coefficient of variation was 13.1% (ESR, 2010).

GLC is also useful for quantifying HCN (Curtis, Grayless & Fall, 2002; Shibata et al., 2004; Murphy et al., 2006). Using a portable gas chromatograph, Curtis, Grayless & Fall (2002) took headspace samples from macerated cassava plants. Reaction of the HCN present with chloramine-T produced cyanogen chloride, which was subsequently chromatographed and quantified using an electron capture detector. Curtis, Grayless & Fall (2002), using a photoionization detector, were also able to detect, in the same samples, the acetone and butanone concomitantly released from the intermediate cyanohydrins (see Figure 2 above) and suggested that the method has utility in screening foodstuffs. The LODs were said to be comparable with those for the traditional colorimetric methods, and the simultaneous detection of cyanide and carbonyls avoids the false positives sometimes encountered with traditional colorimetric approaches. Shibata et al. (2004) developed a headspace GLC method for HCN in blood using a nitrogen–phosphorus detector, as an alternative to the standard colorimetric method, and

achieved an LOD of 0.7–2.4 ng of HCN per millilitre (0.7–2.4 µg/l). Although the method was not applied to foodstuffs, it has the potential to be applied to such samples. Similarly, a sensitive, automated headspace isotope dilution gas chromatography–mass spectrometry method has been developed for cyanide in whole blood that also has the potential to be applied to headspace analysis of cyanogenic foodstuffs (Murphy et al., 2006).

Several methods for analysing HCN by HPLC have also been developed (Sano, Takimoto & Takitani, 1992; Chadha, Lawrence & Ratnayake, 1995; Sumiyoshi, Yagi & Nakamura, 1995). An HPLC–electrochemical detector method for quantifying cyanide produced by homogenizing flaxseed (linseed), with an LOD of 1 mg of HCN per kilogram (signal to noise ratio of 10), has been developed (Chadha, Lawrence & Ratnayake, 1995). Sumiyoshi, Yagi & Nakamura (1995) used HPLC and post-column derivatization with *o*-phthalaldehyde to determine cyanide in environmental river water samples with a recovery of 99.0% (n = 6), a standard deviation of 2.5% (based on peak area) and a linear determination range from 2.5 ng to 1 µg of HCN per millilitre (2.5 µg to 1 mg of HCN per litre). The LOD was 0.1 ng/ml (0.1 µg/l). The applicability of the latter method to HCN in foodstuffs has not been examined.

Several approaches to developing biosensors for HCN quantification have also been investigated. Biosensors, using an immobilized cyanidase (EC 3.5.5.1) that converts cyanide to formate and ammonia and detection of the latter using an ammonia-specific electrode or a pH-sensitive electrolyte/insulator/semiconductor layer structure made of Al/p-Si/SiO_2/Si_3N_4, have been developed and found to have an LOD in the micromole per litre range. An LOD using the ammonia gas electrode was 6 µmol/l, corresponding to 0.16 mg of HCN per litre (Keusgen et al., 2004). A biosensor based on measuring the formate formed from HCN by cyanidase has also been developed and assessed (Mak et al., 2005).

(c) General conclusions

An effective total HCN quantification approach, such as the acid hydrolysis/colorimetric method described by Haque & Bradbury (2002) and recently employed in New Zealand to survey total HCN in plant-based foods (ESR, 2010), is likely to continue to provide the best indication of total HCN. These methods, especially those using added hydrolytic enzymes and picrate or acid hydrolysis and König reagents, have been significantly refined over time, are straightforward, have appropriate LOQs, have low costs and can be employed both semiquantitatively and quantitatively for both laboratory and field use to meet current Codex MLs and likely future food monitoring requirements (some examples are given in Table 5) (Haque & Bradbury, 2002; ESR, 2010).

The more costly and laboratory instrument–requiring analytical methods (HPLC, GLC) as well as ELISA methods for quantification of individual cyanogenic glycosides are particularly suited to agronomic studies—for example, in selecting low–cyanogenic glycoside plant varieties, studying the effects of the growing environment and food preparation methods on the levels of specific cyanogenic glycosides and studying developmental profiles and tissue distribution of those cyanogenic glycosides. With the possible exception of emerging ELISA methods,

Table 5. Examples of methods for total HCN measurement in cyanogenic plants and food

Methods	LOQ	Reference
Colorimetric methods		
Enzyme–picrate	1 mg/kg	Haque & Bradbury (2002)
Enzyme–picrate	0.1 mg/kg	Bradbury (2009)
Isonicotinic acid/barbituric acid	3 mg/kg	ESR (2010)
Other methods		
GLC/cyanogen chloride	69 µg/l	Curtis, Grayless & Fall (2002)
GLC/nitrogen–phosphorus detector	0.7 µg/l	Shibata et al. (2004)
HPLC/electrochemical detector	1 mg/kg	Chadha, Lawrence & Ratnayake (1995)

they do not appear to offer greater speed, simplicity or cost advantages over colorimetric methods for measuring total HCN.

5. SAMPLING PROTOCOLS

Specific sampling protocols for different foods containing cyanogenic glycosides were not found and need to be developed. In a recent survey of total HCN in plant-based foods conducted in New Zealand (ESR, 2010), at least 500 g of each food item were purchased. In the case of highly homogeneous, high-value foods, such as oils, the sample size was at least 200 g. The solid samples were homogenized in a blender immediately prior to analysis, and 10 g replicates of each food item were used to measure total HCN. In applying the enzyme–picrate and acid hydrolysis methods to a wide range of plants used for food, Haque & Bradbury (2002) used 25–100 mg of freshly macerated or ground samples for each analysis.

6. EFFECTS OF PROCESSING

The majority of foods containing cyanogenic glycosides are processed before consumption. Processing aims at reducing levels of cyanogenic glycosides, cyanohydrins and the total HCN content of the food. Some processing methods are better than others at reducing the levels of cyanogenic glycosides, cyanohydrins and HCN/CN^-. The final concentrations depend on the level of these substances before processing occurs and also the practices of the individuals processing the foods (Cardoso et al., 2005). This section summarizes processing methods used to prepare foods containing cyanogenic glycosides and includes summaries of cyanogen concentrations in processed commodities, such as cassava flour (Table 6). In this assessment, "processing" includes commercial food manufacturing as well as domestic food preparation.

The processing steps essential for ensuring low levels of HCN in food products have been reviewed (FAO/WHO, 2008b). The majority of information on

Table 6. Summary of studies and reports showing the total HCN content of some cyanogenic glycoside–containing foods (ESR, 2010)

Food(s)	Country/region	Year	Number/type of samples	Mean HCN equivalent concentration (range) (mg/kg)[a]	Reference
Alcoholic beverages	European Union	NS	Wine (1) Spirits, other (30) Fruit brandy (685)	0.15 0.3 (maximum 5.0) 1.8 (maximum 70)	EFSA (2007b)
Almond, bitter	United States of America	NS	5	4690	Shragg, Albertson & Fisher (1982)
Almond, bitter	NS	NS	Seed Oil	300–3400 800–4000	EFSA (2004)
Almond products	NS	NS	Ground almonds Almond paste Marzipan and related products Marzipan novelties Chocolate enrobed marzipan Almond-containing confectionery	1.4 3.0 5–50 <0.8 1.3 Up to 40	EFSA (2004)
Apple seed	Australia	NS	1	690	Haque & Bradbury (2002)
Apple seed	United States of America	NS	1	566	Kupchella & Syty (1984)
Apricot kernel	NS	NS	NS	120–4000	EFSA (2004)
Apricot pits (kernels)	NS	NS	NS	89–2170	IPCS (2004)
Bamboo shoots	Brazil	NS	Bamboo shoots Canned, sterilized bamboo shoots	894 27	Ferreira et al. (1995)
Bamboo shoots	NS	NS	Immature shoot tip	7700	IPCS (2004)

Food(s)	Country/region	Year	Number/type of samples	Mean HCN equivalent concentration (range) (mg/kg)[a]	Reference
Bamboo shoots	Australia	NS	3	Tip, middle, base: 920, 730, 114 1040, 620, 280 1460, 1140, 380	Haque & Bradbury (2002)
Bamboo shoots	India	NS	NS	551	Chandra et al. (2004)
Bamboo shoots	India	NS	*Dendrocalamus strictus* *Bambusa tulda* *Bambusa vulgaris* *Bambusa balcoa*	386 77[b] 200 67[b]	Satya et al. (2009)
Bamboo shoots (fresh)	India	NS	*Dendrocalamus strictus* - 1 week - 2 weeks - 3 weeks *Bambusa tulda* - 1 week - 2 weeks - 3 weeks *Bambusa bambos* - 1 week - 2 weeks - 3 weeks *Bambusa balcoa* - 1 week - 2 weeks - 3 weeks	Tip, middle, base: 1164, 974, 857 943, 1012, 380 911, 1132, 281 746, 455, 331 564, 629, 302 391, 1061, 280 859, 371, 223 504, 678, 171 422, 997, 139 1006, 811, 304 659, 890, 293 445, 944, 188	Haorongbam, Elangbam & Nirmala (2009)

Table 6 (contd)

Food(s)	Country/region	Year	Number/type of samples	Mean HCN equivalent concentration (range) (mg/kg)[a]	Reference
Cassava	Typical levels	NS	Sweet Bitter	10–20 15–1120	FAO/WHO (2008b)
Cassava	Australia	NS	Tuber, different cultivars	5–67	Bradbury, Egan & Bradbury (1999)
Cassava	Australia	NS	Tuber	27[c]	Haque & Bradbury (2002)
Cassava	Cameroon	NS	Tuber, different cultivars Cassava products - Bâton de manioc - Fufu	197–951 2.5–6.4 13.0–27.5	Agbor-Egbe & Lape Mbome (2006)
Cassava	Ghana	NS	Fresh cassava Akyeke (fermented product, steamed)	69.3, 100.3[d] 1.4, 2.8	Obilie, Tano-Debrah & Amoa-Awua (2004)
Cassava	Malawi	NS	Tubers Sweet (132) Bitter (360)	 29 (1–123) 153 (22–661)	Chiwona-Karltun et al. (2004)
Cassava	Malawi	1996	Tubers Sweet (62) Bitter (170)	 30 (15–93) 153 (43–251)	Mkumbira et al. (2003)
Cassava	Nigeria	NS	NS	153, 127, 68, 65 in four regions	Oluwole (2008)
Cassava	Nigeria, United Republic of Tanzania	2004	Sweet (114) Bitter (20)	105 (8–1064)[d] 103 (27–543)	Oluwole et al. (2007)
Cassava	Nigeria	NS	Raw cassava Ijapu (steeped cassava slices)	76.1 11.8	Sokari & Karibo (1992)

Food(s)	Country/region	Year	Number/type of samples	Mean HCN equivalent concentration (range) (mg/kg)[a]	Reference
Cassava	Indonesia	1996	Tubers (27)	19 (SD = 14)	Djazuli & Bradbury (1999)
Cassava	Indonesia	NS	NS	23.7–50.7	FAO/WHO (2008b)
Cassava	United Republic of Tanzania	NS	Makopa (sun-dried tuber pieces)	9.4 (0–79)	Mlingi et al. (1998)
Cassava flour	Mozambique	1999	119	41 (26–57)	Ernesto et al. (2000); Cardoso et al. (2005)
Cassava flour	United Republic of Tanzania	1993	3	83.7	Carlsson et al. (1999)
Cassava products	Nigeria	NS	Garri (30) Fufu (30) Tapioca (30)	25.4 ± 5.0 (range 20–30) 20.0 ± 6.0 (range 10–30) 17.5 ± 6.2 (range 10–20) (total cyanogens)	Adindu, Olayemi & Nze-Dike (2003)
Cassava products	Democratic Republic of the Congo	NS	Cossettes; 5 different areas, 3 samples each	2.8 ± 0.4 1.7 ± 0.2 1.5 ± 0.2 1.6 ± 0.4 2.9 ± 0.5 (total cyanogens, dry weight)	Ngudi, Kuo & Lambein (2002)
Cassava products	Indonesia	NS	NS	0.4–16.2	FAO/WHO (2008b)
Cassava products	Indonesia	1996	Starch and related products (22) Flour, chips, gaplek (29)	5 (maximum = 19) 54 (SD = 51)	Djazuli & Bradbury (1999)
Cassava products	Australia	2008	Cassava chips/crisps (300)	55.8 (<10–145)	FSANZ (2009)

Table 6 (contd)

Food(s)	Country/region	Year	Number/type of samples	Mean HCN equivalent concentration (range) (mg/kg)[a]	Reference
Cherry stone	United States of America	NS	1	1186	Kupchella & Syty (1984)
Flaxseed	Canada	1987–1989	60	302 (238–373)	Oomah, Mazza & Naschuk (1992)
Flaxseed	Canada	NS	NS	375[e]	Wanasundara, Shahidi & Brosnan (1999)
Flaxseed	Canada	NS	28	590 (17–6500)[f]	Kobaisy, Oomah & Mazza (1996)
Flaxseed/linseed	Australia and New Zealand	NS	Seed, whole (4) Ground seed, meal (15)	2–120 140–370	Haque & Bradbury (2002)
Flaxseed/linseed meal	NS	NS	NS	530	Gupta (1987)
Flaxseed products	Canada	NS	Bread mix Bread (fresh) Energy drink Muffin mix Stabilized flax Waffle mix	6 9 78 3 177 8	Shahidi & Wanasundara (1997)
Lima beans	Nigeria	NS	18	415 (265–530)	Ologhobo, Fetuga & Tewe (1984)
Lima beans	NS	NS	Normal Samples incriminated in poisonings	144–167 2100–3120	Gupta (1987)

Food(s)	Country/region	Year	Number/type of samples	Mean HCN equivalent concentration (range) (mg/kg)[a]	Reference
Lima beans	NS	NS	Java (coloured) Puerto Rico (black) Burma (white)	3000 2900 2000	IPCS (2004)
Lima beans	Philippines	NS	NS	0–10	Laurena, Revilleza & Mendoza (1994)
Lima beans	NS	NS	Seed White American variety Burma variety Black Puerto Rican variety	200 100 2000 3000	EFSA (2004)
Peach kernel	NS	NS	NS	470	EFSA (2004)
Peach kernel	United States of America	NS	1	1451	Kupchella & Syty (1984)
Sorghum (whole immature plant)	NS	NS	NS	2400	IPCS (2004)
Soya bean hulls	NS	NS	NS	1.24	IPCS (2004)
Soya protein products	NS	NS	NS	0.07–0.3	IPCS (2004)
Stone fruit juices	NS	NS	Cherry juice, commercial Cherry juice, home-made, pitted fruit Cherry juice, home-made, pits included Apricot juice Prune juice	4.6 5.1 23 2.2 1.9	IPCS (2004)

Table 6 (contd)

Food(s)	Country/region	Year	Number/type of samples	Mean HCN equivalent concentration (range) (mg/kg)[a]	Reference
Stone fruit kernels	Australia	NS	Peach (1) Plum (1) Nectarine (1) Apricot (1)	710 696 196 785	Haque & Bradbury (2002)
Stone fruit products	NS	NS	Cherry juice Plum juice Apricot juice Peach juice Stone fruit preserves Canned stone fruit Stone fruit spirits	0.5–12 0.3–1.0 0.3–7.8 2.3–5.9 ~0.2 Up to 4 <10	EFSA (2004)

NS, not specified

[a] Results on a fresh weight basis, unless otherwise stated.

[b] Stored at –18 ºC for 2 months.

[c] Samples were analysed by two different methods. Results reported here are from the acid hydrolysis method used in ESR (2010).

[d] Dry weight basis.

[e] Sum of linustatin and neolinustatin.

[f] Flaxseed samples were analysed for total cyanide by three different methods. As no significant difference was found in the cyanogen content obtained by any method, only one data set is provided (barbituric acid–pyridine reagent method).

the effects of processing on levels of cyanogenic glycosides in foods was found in the literature and reviewed. Some data submitted by Australia and New Zealand were also reviewed.

As indicated previously, the mechanism by which the reduction of cyanogenic glycosides occurs via processing is the disruption of the cells (e.g. in grating or pounding), releasing the cell contents and allowing the cyanogenic glycosides to come into contact with glucosidase enzymes (Montagnac, Davis & Tanumihardjo, 2009), as shown in Figure 2 above. Heat applied during boiling accelerates the loss of HCN (Ngudi, Kuo & Lambein, 2003). Soaking allows the natural enzymes to convert the cyanogenic glycosides present to glucose and cyanohydrins, which, at pH values above 5, release HCN gas, which disperses slowly and harmlessly (FSANZ, 2008a).

Traditional methods of cassava preparation, such as soaking (Kendirim, Chukwu & Achinewhu, 1995; Agbor-Egbe & Lape Mbome, 2006), sun drying (Nambisan & Sundaresan, 1985; Mlingi & Bainbridge, 1994), fermentation (Tuncel et al., 1990; Kendirim, Chukwu & Achinewhu, 1995; Obilie, Tano-Debrah & Amoa-Awua, 2004), storage (Onabolu, Oluwole & Bokanga, 2002) and cooking (Ferreira, Yotsuyanagi & Carvalho, 1995; Ngudi, Kuo & Lambein, 2003; Agbor-Egbe & Lape Mbome, 2006), all lead to significantly reduced levels of total potential for dietary HCN exposure (Padmaja, 1995; Montagnac, Davis & Tanumihardjo, 2009). There is, however, a direct correlation between the total HCN content in the tuber being processed and the final level of total HCN achieved (Cardoso et al., 2005), so there is a continuing need to develop cultivars displaying good agronomic potential and low levels of cyanogenic glycosides (Dixon, Asiedu & Bokanga, 1994). It is also important to establish food processing methods that ensure efficient hydrolysis of cyanogenic glycosides to cyanohydrins, the efficacious conversion of these to HCN and subsequent elimination of the HCN if food products are to achieve Codex MLs (Haque & Bradbury, 2002; Ernesto et al., 2002a; Cardoso et al., 2005; Bradbury, Cliff & Denton, 2011). Reports of transgenic methods of reducing cyanogenic glycoside levels in foods have included inhibiting two cassava cytochrome P450 genes (CYP79 D1 and D2), which are responsible for cyanogen synthesis and accelerating cyanogen turnover or detoxification and volatilization during processing by overexpressing the hydroxynitrile lyase enzyme that catalyses the last step in cyanogenesis, resulting in a 3-fold increase in cyanogen turnover (Siritunga & Sayre, 2007; Nambisan, 2011).

In regard to the effectiveness of processing of cassava, Haque & Bradbury (2002) developed a colorimetric method for selectively analysing the amounts of 1) linamarin, 2) acetone cyanohydrin and 3) HCN in gari samples and showed that, under some conditions of processing, samples of gari can contain unacceptably high levels of acetone cyanohydrin. This intermediate substance is known to be readily broken down to HCN in the weakly alkaline conditions of the intestine (Mlingi et al., 1995), so that gari with high acetone cyanohydrin levels represents a particular threat to consumers, much greater than from residual linamarin, which, in the absence of suitable hydrolytic enzymes, is less effectively converted to HCN in vivo (Carlsson et al., 1999). Thus, during processing, the effectiveness of conversion of cyanogenic glycosides to cyanohydrins, their subsequent conversion

to HCN and its volatilization are particularly important to ensuring the safety of the final product. Conditions of pH at various stages of processing are likely to be important for effective detoxification. The enzymatic hydrolysis requires neutral pH conditions, the breakdown of the cyanohydrin to release HCN is ideally achieved under alkaline conditions, and the volatilization of the HCN will occur best at acidic pH values.

6.1 Cassava tubers

There are many methods for the preparation of cassava used around the world. These methods are summarized and compared below.

Cassava tubers are highly perishable and deteriorate in 3–4 days once harvested. Plants are usually left in the ground until needed for food processing or consumption. Cassava is consumed in a number of forms: flour used for cooking, tuber slices, tuber chips/crisps, baked grated tuber, steamed grated tuber, pan-fried grated tuber, steamed whole tuber and tapioca pearls made as a pudding.

Bitter cassava has more cyanogenic glycosides and has a bitter taste; sweet cassava has lower amounts of cyanogenic glycosides. There are large amounts of cyanogenic glycosides in the tuber cortex (skin layer) and generally smaller amounts in the tuber parenchyma (interior). This is why peeling and cooking (roasting, boiling or baking) of the tuber flesh are generally effective ways of removing cyanide from sweet cassava (Cardoso et al., 2005). Bitter varieties of cassava require more extensive processing to achieve Codex MLs and may only reach these levels at a point at which the nutritional value of cassava has been significantly reduced.

Cardoso et al. (2005) developed and applied a simple equation of total cyanide content of cassava tuber to different methods of food preparation and processing and showed that sun drying and heap fermentation result in a high retention of cyanide in flour compared with soaking and crushing prior to sun drying or roasting. The former methods can leave up to 30% of the original total HCN in cassava flour, whereas in the latter case, only 3% of the original total HCN remains. This has implications for whether bitter cassava with total HCN above particular levels can ever achieve Codex MLs in food products.

Soaking involves immersing peeled tubers in water for several nights. This allows good contact between the water-soluble cyanogenic glycosides and glycosidases. Shorter soaking can lead to cyanide intoxication. Soaking followed by sun drying, pounding and sieving has been shown to be a very effective method for removing cyanide from cassava (Bradbury, Cliff & Denton, 2011).

Sun drying involves drying peeled whole tubers or large pieces in the sun. Heap fermentation is more labour intensive and time consuming. It involves leaving peeled and cut tubers in a small heap for 3–5 days during which some fermentation takes place, with liberation of HCN. The tubers are then sun dried and can be pounded and sieved to produce flour. Heap fermentation results in a greater reduction of cyanogenic glycosides compared with sun drying because of the fermentation process in which microflora break down the cyanogenic glycosides, and usually a greater length of time is involved to allow cyanogenic glycoside breakdown to occur (Cardoso et al., 2005; FSANZ, 2008a; Cliff et al., 2011).

Processing methods that use grating and crushing are more effective in removing cyanide than sun drying because they allow more contact between cyanogenic glycosides and the hydrolysing enzyme(s), which promotes rapid breakdown to HCN gas, which escapes into the air. Sun drying does not break down the flesh as much or disrupt the tissues, so that the cyanogenic glycosides stay intact (Cardoso et al., 2005). Pounding alone can reduce the cyanogenic potential by 60–70% (Bokanga et al., 1994).

Boiling may not be the most effective method of removing cyanide, with only around 50% of it being removed. The linamarase enzyme, which breaks down linamarin to acetone cyanohydrin, is denatured at 100 °C. Levels of cyanogens and free cyanide in boiled products are low (Montagnac, Davis & Tanumihardjo, 2009).

Linamarin, the main cyanogenic glycoside in cassava, is more stable in neutral or weak acid conditions and at temperatures up to 100 °C (Bradbury, Egan & Lynch, 1991). Processing that includes high pH values (>5) and high temperatures facilitates the breakdown of acetone cyanohydrin (Fokunang et al., 2001), which is stabilized at lower pH levels in products such as gari.

Gari (West Africa) is prepared by storing peeled, grated cassava in a wet bag for 2–3 days. The damp mash is roasted in a metal pan over a fire to remove HCN and moisture, producing a hard, crunchy product with about 20 mg/kg of HCN. Typical concentrations of total HCN reported are 0–32 mg/kg over 200 samples (Oke, 1994) and 1–39 mg/kg (Aletor, 1993).

Although gari generally has a much lower measured total cyanide content than cassava flour, the main cyanogen present in gari (pH 4.1) is acetone cyanohydrin, whereas linamarin is the main cyanogen in cassava flour (pH 6.5) (Bradbury, 2009). Acetone cyanohydrin is stable at low pH values; it readily releases HCN in the weakly alkaline environment of the intestines (Mlingi et al., 1995). It is a cyanogen capable of releasing all its HCN following ingestion, whereas the linamarin in cassava flour is estimated to release only 50% of its HCN in vivo (Carlsson et al., 1999; Bradbury, 2009). Thus, the total HCN values recorded for gari and cassava flour do not reflect equal toxicities. Perhaps as a consequence, gari has a Codex ML of 2 mg/kg for total HCN, whereas the Codex ML for total HCN in cassava flour is 10 mg/kg.

Cossettes are generated when bitter cassava tubers are soaked and fermented for 3 days, then sun dried. This is one of the forms that can be stored for later use or traded. In one study (Ngudi, Kuo & Lambein, 2002), all cossettes were below the Codex ML of 10 mg/kg in flour.

In the South Pacific, including Papua New Guinea and Fiji, the introduced cassava varieties are virtually all sweet, and, after peeling, the tubers may be boiled and safely eaten. In some parts of the Pacific, cassava tubers are placed in shallow pits surrounded by damp sawdust, and the pit is then covered with soil, preserving the tubers for several months (FSANZ, 2004b).

During periods of drought, the cyanide content of both sweet and bitter cassava varieties increases (Bokanga et al., 1994). Bitter cassava varieties are more drought resistant and therefore more readily available and cheaper. Heap

fermentation is preferred over sun drying to eliminate more cyanide in bitter cassava (Ernesto et al., 2002a; Bradbury, Cliff & Denton, 2011). However, owing to food shortage in times of drought, less time is available for the additional processing required for heap fermentation (FSANZ, 2004b).

Some processing methods are undertaken to produce a form more able to be transported and stored, which may include dried minimally processed cassava in the form of pellets/chips, slices or gari (Vetter, 2000; FSANZ, 2004b). These forms can then be utilized when needed by being processed further in the preparation of other foods. Drying may cause cyanogen concentrations in cassava products to be higher, up to 2.5 times (Cardoso et al., 2005). Packaging for transport also plays a role in the reduction of cyanogenic glycosides. For example, transport of cossettes in jute sacks appears to allow continued reduction of cyanogenic glycosides, whereas airtight wrapping prevents the release of cyanide (Ngudi, Kuo & Lambein, 2002).

6.2 Cassava flour

HCN levels are generally low in cassava products, because HCN rapidly dissipates or is dissolved and removed in processing water. However, high levels of cyanogens can remain in flour if the cassava is not properly processed, especially if the flour has a high moisture content and low pH (Carlsson et al., 1999).

The process of using sun drying to produce flour involves peeling the cassava tuber, drying it or slices of it in the sun for about 7 days, pounding it with a wooden pestle and mortar, then sieving to give a white, powdered product. The production of cassava flour by heap fermentation involves a small heap of peeled tubers left in the shade for about 4 days. The tubers are then sun dried, pounded and sieved to give slightly coloured flour (Djazuli & Bradbury, 1999; Bradbury, Cliff & Denton, 2011).

There are many “porridges” (often referred to as stiff porridge) made from cassava flour, usually by mixing flour with boiling water. Examples of these are ugali and luku.

Cassava flour produced by heap fermentation has a lower cyanide content compared with cassava flour produced by sun drying. Sun drying can leave high amounts of linamarin and low levels of HCN (Carlsson et al., 1999). One study by Cardoso et al. (2004) found that heap-fermented flour contained an HCN concentration of 17 mg/kg, around half the total HCN content of sun-dried flour (HCN concentration of 30 mg/kg). Ernesto et al. (2000) also showed a 50% lower concentration of total cyanogens from heap fermentation compared with sun drying. A study in Mozambique showed that flour produced via sun drying had total cyanide retention of 25–33%, whereas heap fermentation retained 12.5–16.5% (Cardoso et al., 2005). No significant difference has been found between milling or pounding to produce cassava flour in reducing cyanogen content (Mlingi et al., 1998).

A simple wetting method that reduces the cyanide content of cassava flour 3- to 6-fold has been developed and proven (Bradbury, 2006; Bradbury & Denton, 2010a,b), but it is not being widely used, even in countries where chronic cyanide poisoning occurs (Bradbury, Cliff & Denton, 2011).

In times of low rainfall and drought, total cyanide content in flour increases. The average total cyanogenic potential in flour in normal years is 40–46 mg of total

HCN equivalents per kilogram of flour (fresh weight) (Cardoso et al., 1998), and in years of low rainfall, more than 100 mg of HCN equivalents per kilogram of flour (Cardoso et al., 2005). Under conditions of low rainfall, total cyanide concentrations in 16–26% of flour samples fall below 40 mg/kg (Indonesian limit), compared with 51–67% in an average year (Cardoso et al., 2005). Distribution curves of total cyanide levels in flour in Mozambique show that the percentage of samples exceeding total cyanide concentrations of 100 mg/kg increased from 6% in an average year to 43–65% in a low-rainfall year (Cardoso et al., 2005).

Cassava flour in Mozambique has been shown to contain levels of both available cyanogens (acetone cyanohydrin ± HCN/CN^-) and partially available cyanogens (linamarin). For Nampula City ($n = 12$ samples), mean levels of acetone cyanohydrin ± HCN/CN^- were 13 ± 9 mg/kg, and for Nametil town ($n = 25$ samples), 17 ± 12 mg/kg. Levels of linamarin were 17 ± 14 mg/kg for Nampula City and 41 ± 57 mg/kg for Nametil town. Depending on additional changes in these levels when the flour is further processed, dietary exposure to both of these components could occur (Ernesto et al., 2000). In another study, Tylleskär et al. (1992) showed that the cyanohydrins are the main source of cyanide in flour made from insufficiently soaked cassava tubers. They also reported that cyanohydrins disappear during storage of the product, most likely due to slow elimination and loss of resultant volatile HCN.

Sulfur-containing amino acids (methionine and cysteine) are used by the body to help detoxify cyanide released from cyanogenic glycosides. Soaking for several days changes the nitrogen content and therefore the amino acid profile of the flour (Cardoso et al., 2004). Sun drying and heap fermenting the cassava tubers for processing into flour are unlikely to change the amino acid content (Cardoso et al., 1998). This would be a preferred outcome, so the intake of sulfur-containing amino acids is maximized to assist in reducing the likelihood of toxicological effects from exposure to cyanogenic glycosides.

6.3 Cassava starch

The process used to obtain cassava starch is effective at removing cyanogens. Montagnac, Davis & Tanumihardjo (2009) reported total cyanogen removal during the production of cassava starch. The production of starch involves using peeled, shredded cassava tubers. These are covered in cloth and pressed while washing. The water is left for the starch to settle, then the water is discarded and the starch dried and ground (Djazuli & Bradbury, 1999).

Cassava starch has lower HCN levels (mean 5 ± 4 mg/kg; maximum 19 mg/kg) than do cassava chips (slices), flour and gaplek (sliced, dried root of cassava) (mean 54 ± 51 mg/kg) (Djazuli & Bradbury, 1999).

6.4 Cassava leaves

There are high levels of cyanogens in cassava leaves (Cardoso et al., 2005), which can be an important part of the diet. They are consumed as a standard vegetable in many countries (e.g. Sierra Leone, Guinea, the Democratic Republic of the Congo and the Central African Republic). Young emerging leaves contain the highest levels of cyanogenic glycosides, with older leaves having 50–70% lower

levels (Nambisan, 2011). Cassava leaves have 10 times more cyanogens than cassava tubers (Montagnac, Davis & Tanumihardjo, 2009).

A traditional method of processing cassava leaves is pounding and cooking/boiling (Cardoso et al., 2005), which results in considerable detoxification, removing about 99% of the cyanogens (Ngudi, Kuo & Lambein, 2003; Montagnac, Davis & Tanumihardjo, 2009). There are other methods of preparation of the cassava leaves, including a relish made from them called mpondu (prepared by blanching young leaves followed by grinding and extensive boiling) (Simons-Gérard et al., 1980) or saka-saka (Banea-Mayambu et al., 2000). They can also be used as a sauce and served with stiff porridge that is made from tuber flour (Cliff et al., 1997).

Ngudi, Kuo & Lambein (2003) studied residual cyanogens before and after cooking commercially pounded cassava leaves. The raw leaves were up to 10 times higher in cyanogens than the cooked leaves. Cooking removed 96–99% of total cyanogens. Of the five areas sampled, total cyanogen levels for raw samples ranged from 35.9 ± 0.4 to 107.5 ± 0.8 mg of HCN equivalents per kilogram dry weight; in cooked samples, the levels ranged from 0.3 ± 0.04 to 1.9 ± 0.2 mg of HCN equivalents per kilogram dry weight. Levels of acetone cyanohydrin ranged from 5.7 ± 1.9 to 24.2 ± 4.5 mg of HCN equivalents per kilogram dry weight in raw samples, and acetone cyanohydrin was not detected in any cooked samples. Linamarin levels ranged from 30.2 ± 2.4 to 83.4 ± 5.3 mg of HCN equivalents per kilogram dry weight in raw samples and from 0.3 ± 0.04 to 1.9 ± 0.2 mg of HCN equivalents per kilogram dry weight in cooked samples.

6.5 Bamboo shoots

There are approximately 1200 species of bamboo, although only a small number are used as food. The cyanide content in raw bamboo shoots, up to 1000 mg of total HCN per kilogram (Ferreira et al., 1995) and at 7000 mg of total HCN per kilogram (IPCS, 2004), is reported to decrease substantially following harvesting (FSANZ, 2004b). Cyanogenic glycosides in bamboo shoots can be extracted by cooking or maceration. Boiling can adequately reduce levels of cyanogenic glycosides. The process of canning bamboo shoots frees and removes HCN (FSANZ, 2004a).

The following steps are typically taken in the preparation of bamboo shoots: 1) fresh bamboo shoots are cut in half lengthwise; 2) the outer leaves are peeled away, and any fibrous tissue at the base is trimmed; 3) the bamboo shoot is thinly sliced into strips; and 4) the shoots are boiled in lightly salted water for 8–10 minutes. In Thailand and Viet Nam, shoots can be finely grated and used in salads. In Japan, shoots are sometimes boiled whole for more than 2 hours. The most common preparation involves boiling the shoots in stocks, soups or salted water for use in assorted dishes (FSANZ, 2004b).

Ferreira et al. (1995) studied the effect of cooking bamboo shoots at different temperatures (98, 100 and 122 °C) for 20, 100 and 180 minutes. Results are shown in Table 7. The starting concentration in the apical parts of the bamboo shoots was 894 mg/kg. There was little effect on HCN removal up to 98 minutes at any temperature, but at longer than 123 minutes, there was an inverse relationship between time and temperature, with temperatures nearer to 98 °C being more

Table 7. Percentage of HCN extracted from bamboo shoots based on cooking time and temperature

Cooking time (minutes)	% of HCN extracted		
	98 °C	110 °C	122 °C
20	68.1	70.8	75.1
100	92.7	91.4	94.2
180	96.8	96.9	94.3

Source: Ferreira et al. (1995)

effective at removing HCN. The highest HCN extraction was with cooking for more than 148 minutes. Up to 96% of HCN was lost after about 140 minutes. Optimal cooking conditions for HCN extraction were 148–180 minutes at 98–102 °C. Longer cooking times result in greater extraction.

6.6 Ready-to-eat cassava chips/crisps

Levels of total HCN in ready-to-eat cassava chips/crisps in Australia and New Zealand have been reported from below 10 mg/kg up to 145 mg/kg (FSANZ, 2008a). Ready-to-eat cassava chips/crisps are composed predominantly of cassava, cassava flour or tapioca flour (i.e. cassava products that would be expected to be adequately processed) (FSANZ, 2008a). The amounts of each of these as ingredients in the chips/crisps vary, depending on the manufacturer. Some ready-to-eat cassava chips/crisps could contain total HCN at levels above 10 mg/kg, because they contain dried raw cassava, which may contain higher levels of total HCN than cassava flour. To comply with an ML for total HCN of 10 mg/kg (in Australia and New Zealand) (FSANZ, 2011), these types of chips/crisps need to be produced from cassava with low total HCN levels, or the dried cassava used for them must be further processed to adequately reduce the levels of total HCN. This is achievable, as some chips/crisps already contain less than 10 mg/kg of total HCN (FSANZ, 2008b).

6.7 Beverages and marzipan

The kernels of stone fruits are a source of cyanogenic glycosides, and fruit juices have detectable levels of cyanogenic glycosides. HCN is contained in bound form in the stones of fruits such as bitter apricots, plums and cherries and is released through enzymatic action during the maturation process of the fruit and after the harvest (EFSA, 2007a). Bitter almonds and bitter apricot kernels are among the nuts and fruits used to make cyanogenic glycoside–containing flavours for use in products such as liqueurs and marzipan. From a flavouring point of view, the most important are those liberating benzaldehyde as a flavouring compound—namely, amygdalin, sambunigrin and prunasin. These compounds are essential to the flavours.

Typical HCN levels found in fruit juices, fruit preserves and brandies are as follows: 0.9–12 mg of total HCN per litre in cherry juice; 0.3–1 mg of total HCN per litre in plum juice; 2.3–5.9 mg of total HCN per litre in peach juice; up to 4 mg of total HCN per kilogram in canned stone fruit; and less than 10 mg of total HCN per litre in Kirsch distilled from cherries (EFSA, 2007b).

Marzipan is mostly made from sweet almonds, which are low in amygdalin and for which the preparation procedure should eliminate most of the cyanide (NZFSA, 2008); however, total HCN levels up to 35 mg/kg have been reported, and total HCN levels of 50 mg/kg have been found in "baker's raw" marzipan paste (EFSA, 2007a).

7. PREVENTION AND CONTROL

Annex II of Council Directive 88/388/EEC on flavourings sets the following maximum levels for HCN in foodstuffs and beverages to which flavourings or other food ingredients with flavouring properties have been added: 1 mg/kg in foodstuffs and 1 mg/kg in beverages, with the exception of 50 mg/kg in nougat, marzipan or its substitutes or similar products, 1 mg per per cent volume of alcohol in alcoholic beverages and 5 mg/kg in canned stone fruit. HCN may not be added as such to foodstuffs (CEC, 1988).

8. LEVELS AND PATTERNS OF CONTAMINATION OF FOOD COMMODITIES

Cyanogenic glycoside levels in plants used for food and flavouring can vary greatly (see Table 6 above) according to season, location and soil type, between cultivars of a particular species and due to processing (Annex 1, reference *102*; Bokanga et al., 1994; FAO/WHO, 2008b; Bradbury, Cliff & Denton, 2011; Nambisan, 2011). Oomah, Mazza & Naschuk (1992), for example, identified the cultivar as the most important factor affecting the level of cyanogenic glycosides in the 10 flaxseed cultivars they examined. Total cyanide levels in the tubers of cassava have also been shown to increase substantially in drought years (Bokanga et al., 1994) and to significantly raise the risk of toxic effects occurring (Cardoso et al., 2005; Bradbury, Cliff & Denton, 2011).

Bitter almonds and bitter apricot kernels are among the nuts and fruits used to make cyanogenic glycoside–containing flavours for use in products such as marzipan and liqueurs. From a flavouring point of view, the most important are those liberating benzaldehyde as a flavouring compound—namely, amygdalin, sambunigrin and prunasin. These are essential to the flavours.

Typical concentrations of HCN in raw unprocessed commodities can be found in Table 1 above. See also Table 6 above for further details on concentrations of HCN in unprocessed commodities and on levels found in processed foods.

9. DIETARY EXPOSURE ASSESSMENT

9.1 Introduction and background

Cyanogenic glycosides were previously considered by the Committee at its thirty-ninth meeting in 1992 (Annex 1, reference *101*). No dietary exposure information was considered at that meeting.

Cyanogenic glycosides are found in a variety of different foods, as outlined in section 8 above. One of the major sources is cassava and products made from cassava, with other sources including bamboo shoots, stone fruit kernels, almonds, sorghum and lima beans. Cyanogenic glycosides can also be present in food as a result of using foods containing cyanogenic glycosides as flavouring agents in other foods.

Cassava is a staple food in the tropics, where it is the third most important crop used for food after rice and maize (Boccas, 1987; Banea-Mayambu et al., 2000; Ngudi, Kuo & Lambein, 2003; IPCS, 2004; Bradbury, Cliff & Denton, 2011). It is grown and consumed commonly in Africa, Latin America, Asia and Pacific Island countries and is also exported from many countries in these regions. Both the starchy root and leaves are eaten. Cassava yields well in poor soil without fertilizer and is drought resistant and therefore is a key crop for many communities, in Africa in particular, in times of drought.

Cassava products are commonly consumed, including the use of cassava flour in preparation of a traditional "porridge" and foods more typical of developed countries, such as cookies and cakes (Damardjati, Widowati & Rachim, 1993). Cassava starch/tapioca is also common. Semiprocessed cassava or flour is also used in processed foods, such as ready-to-eat cassava chips/crisps (FSANZ, 2008a).

Cyanogenic glycosides (e.g. linamarin in cassava) are enzymatically hydrolysed to give a cyanohydrin and glucose; then, in a second reaction, the cyanohydrin, which is relatively unstable, breaks down spontaneously above pH 5 or enzymatically to give HCN gas and a ketone (Fokunang et al., 2001; Bradbury, Cliff & Denton, 2011) (also outlined in Figure 2 above). Exposure to HCN through the diet can arise from consumption of intact cyanogenic glycosides or from consumption of cyanohydrins or HCN itself.

Insufficient processing of foods containing cyanogenic glycosides will lead to cyanogenic glycosides remaining in the food. This can be exacerbated in times of drought and food shortage when shortcuts in processing, both the methods used and time taken, for cassava, for example, are taken to ensure that some food is available for a family to consume.

The level of dietary exposure to cyanogenic glycosides depends on the initial level of cyanogenic glycosides, cyanohydrin or HCN in the food, the effectiveness of processing in reducing these levels and the amount of food consumed. The dietary exposure, along with the nutritional status of the individual, including the consumption of sulfur-containing amino acids, metabolic conversion and the ability of the individual's body to detoxify the cyanogenic glycosides, will determine whether any adverse effects occur.

Higher dietary exposures tend to occur at specific times, such as during the cassava harvest and during drought, when less variety of food is available and bitter cassava varieties are more commonly available, more heavily relied upon as a food source and generally less effectively processed to remove cyanide (Cliff et al., 1985, 2011).

Production and consumption of cassava are increasing (Bradbury, Cliff & Denton, 2011; Nyirenda et al., 2011); therefore, there is a greater potential for dietary exposure to cyanogenic glycosides in the human population, particularly if introduced into areas that have no experience in preparation methods that reduce cyanide.

9.2 Methods

The Committee evaluated dietary exposure to cyanogenic glycosides, including a limited amount of information that was submitted to the Committee. Dietary exposure estimates were submitted for Australia and New Zealand. Other information was found in the literature, particularly from African countries and some from Europe.

Estimates of dietary exposure to cyanogenic glycosides have been primarily expressed as exposure to total HCN, because this was the form recorded in most of the occurrence or analytical data. However, dietary exposure can be to cyanogenic glycosides, cyanohydrins or HCN, depending on the processing of the food. The estimated dietary exposures to total HCN are a worst-case scenario, because it is assumed that 100% of the cyanogenic glycosides are extracted and converted to HCN.

The dietary exposure estimates have been determined in a variety of ways. Different types of consumption data (e.g. individual dietary records, food balance sheets and food frequency questionnaires) have been used, along with different types of concentrations (e.g. maximum levels in food regulations and analysed concentrations) and different methodologies. Some estimates of dietary exposure are presented as per capita, and some are only for consumers of HCN.

Based on the properties and possible toxicological effects of cyanogenic glycosides, estimates of both acute (short-term) and chronic dietary (long-term) exposure have been evaluated. This is because of the potential for both acute and subchronic effects to occur. Generally, acute dietary exposure estimates are conducted using a high percentile level of consumption for a food and a high HCN concentration for that food. Exposure from one meal or for 1 day is of relevance. Exposures are usually assessed for each food individually. Chronic dietary exposure estimates can include a range of foods and are usually based on mean or median concentrations in each food, because usual exposure over a longer period of time is of relevance for chronic assessments.

Both deterministic and probabilistic estimates of dietary exposure have been reviewed for HCN. A deterministic or “point” estimate is a single value for the estimate of dietary exposure. For example, this can be a mean exposure for a population or population subgroup derived by multiplying a mean concentration of a food chemical by the mean consumption of that food. A probabilistic assessment results in a distribution of estimated dietary exposures, where at least one input (consumption and/or concentration data) is derived from a distribution of values (either actual or simulated distributions).

More detailed information on conducting dietary exposure assessments can be found in FAO/WHO (2009b) and WHO (2011a) for acute dietary exposure assessments.

Cyanogenic glycosides are converted to other chemicals in the body. These metabolites are commonly used as indicators of the levels of dietary exposure to certain substances or consumption of certain foods. There were many papers in the literature that focused on quantifying these biomarkers of exposure (e.g. serum or urinary thiocyanate, a cyanide metabolite). The biomarkers of relevance to dietary exposure and the influence of food consumption patterns on levels of biomarkers have been outlined below, and the results from relevant papers summarized. The estimates of dietary exposure have not been converted in any way or had any factors applied that take into account the proportion of cyanogenic glycosides either excreted from the body unchanged or metabolized in the body.

In dietary exposure assessments, exposures are usually estimated for the population as a whole as well as for population subgroups that may be more susceptible to exposure to a particular substance or could have higher exposures than the general population and could be more likely to show adverse effects. No particular vulnerable population subgroups were determined by the Committee as requiring separate dietary exposure assessments to be conducted and/or evaluated.

There are, however, some population subgroups with the potential for higher exposure to cyanogenic glycosides or cyanide. These include individuals involved in large-scale processing of cassava and those consuming significant quantities of improperly prepared foods containing cyanogenic glycosides (e.g. cassava, apricot pits and bitter almonds). Cigarette smokers have a high exposure to HCN. Certain conditions that arise from consuming inadequately processed cassava tend to affect certain age/sex groups. Konzo may be more common in children over 2 years of age and women of childbearing age (Cardoso et al., 2004; Cliff et al., 2011). Children tend to have higher exposures to food chemicals on a body weight basis due to their high food consumption per kilogram of body weight and their lower body weights. Some exposure estimates have included children separately, so these have been reviewed where provided. The chronic condition tropical ataxic neuropathy occurs in older people who consume a cassava-based diet. Also, the effects of dietary exposure to cyanogenic glycosides are exacerbated in undernourished populations (i.e. where consumption of sulfur-containing amino acids, which assist in detoxifying cyanogenic glycosides in the body, is insufficient). Cyanide exposure from cassava exacerbates goitre and cretinism in iodine-deficient areas, so populations with iodine deficiency disorders can be affected (Lagasse et al., 1980; Delange, Ekpechi & Rosling, 1994; Peterson et al., 1995). A condition described as “malnutrition diabetes” found in the tropics is also linked to the consumption of cassava and other foods containing cyanogenic glycosides in malnourished individuals (McMillan & Geevarghese, 1979).

It is difficult to undertake specific dietary exposure estimates for population groups such as the undernourished or iodine deficient. This is because many national dietary surveys do not have this level of detail on survey respondents, there may be insufficient respondents in this subpopulation group to ensure robust results or individual studies may not collect this information from subjects. For an assessment such as this for cyanogenic glycosides, this information would be useful, given that cassava consumption, for example, tends to be more common in areas of the world where malnutrition is more prevalent.

The exposure estimates or studies on biomarkers of exposure reviewed did include both adults and children. In addition, some of the literature included study groups from poorly nourished areas or those suffering from drought (e.g. in some areas in Africa). Where information on population groups of relevance was available, this has been reviewed and the relevant population groups identified.

As processing of foods containing cyanogenic glycosides is an important determinant of the level of dietary exposure, the effects of processing have been considered where available.

Owing to limitations with the available consumption and concentration data, no international estimates of either chronic or acute dietary exposure were assessed. This is discussed further in section 9.3.2.

There are some MLs established by the Codex Alimentarius Commission and some other countries for HCN in certain foods. Therefore, where available, dietary exposure estimates using MLs were reviewed. Using the MLs represents the upper level of total HCN likely to be found in foods in trade. Also, if foods are adequately processed, they should contain HCN below these levels. Estimates of dietary exposure based on MLs have been conducted using consumption data from the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diets and the WHO large portion data.

The majority of people are exposed to very low levels of cyanide from both naturally occurring and anthropogenic sources—from food (both naturally occurring and through the use of flavouring agents added to food), water, air, dust and cigarette smoke (IPCS, 2004). Ideally, all sources of exposure to a substance should be considered in order to determine total exposures and the risk to human health. Primarily the exposures to cyanogenic glycosides from food are considered below. Although cyanide is also present in drinking-water, the exposure estimates evaluated did not include water. Some estimates from occupational exposure have been documented from workers in cassava processing plants, including the evaluation of biomarkers of exposure. Information on the levels of exposure from sources other than food has been reviewed and summarized where possible.

9.3 Estimates of dietary exposure

9.3.1 National estimates of dietary exposure

National dietary exposure estimates are summarized for a limited number of countries. These included dietary exposure estimates submitted for Australia and New Zealand. Other dietary exposure estimates were found in the literature for African and European countries.

(a) Australia

Consumption data for both cassava and bamboo shoots in Australia were submitted to the Committee. The proportion of consumers and amounts consumed are low (Table 8). In Australia, there is limited use of cassava roots, but processed products, such as cassava chips/crisps and tapioca, are widely available.

Table 8. Consumption of cassava and bamboo shoots for Australia from national nutrition survey data

Food	Population group	Number of consumers[a]	Consumers as a % of total respondents[b]	Mean consumption, consumers only (g/day)[c]
Cassava	Children 2–16 years	628	14.0	5
	Adults 17+ years	13	0.1	34
Bamboo shoots	Children 2–16 years	256	5.7	2
	Adults 17+ years	55	0.5	23

[a] Total number of respondents: 17+ years = 11 129; 2–16 years = 4487.
[b] Based on individual dietary records from a single 24-hour recall for 17+ years (1995 National Nutrition Survey) and two 24-hour recalls for 2–16 years (2007 National Children's Nutrition and Physical Activity Survey).
[c] Includes where these foods are used as ingredients in mixed foods or dishes.

Fresh bamboo shoots in significant quantity have been available in Australia only since 2001, but the market is reported to be expanding (FSANZ, 2004b). Bamboo shoots are popular in Asian-style dishes.

(i) Acute dietary exposure estimates

In a simple deterministic calculation conducted for ready-to-eat cassava chips/crisps, it was estimated that a 200 g serving (two packets of chips/crisps) containing linamarin at a level equivalent to 80 mg/kg of available HCN eaten by a 20 kg child in a 2-hour sitting, assuming that 20% of the HCN from ingested linamarin is absorbed, results in a dietary exposure to HCN of 3000 µg/day or 150 µg/kg bw (FSANZ, 2008a). Additionally, 1 g of linamarin can release a maximum of 109.3 mg HCN; therefore, if the chips/crisps contain HCN at 80 mg/kg, this was equated in the report to a maximum linamarin concentration of approximately 800 mg/kg. So, in 200 g of cassava chips/crisps, a child would consume 160 mg (or 8 mg/kg bw) of linamarin.

A more detailed assessment for ready-to-eat cassava chips/crisps for Australia was subsequently conducted (FSANZ, 2008b) based on consumption of crisps (e.g. potato), "extruded snacks" and other salty snacks as a proxy for ready-to-eat cassava chips/crisps. This was because the most recent nutrition survey available at the time in Australia (the 1995 National Nutrition Survey) did not contain specific consumption data for ready-to-eat cassava chips/crisps. Although this proxy may result in a higher proportion of the population consuming cassava chips/crisps, it is a reasonable approximation of the amount that would be consumed and therefore a reasonable estimate of the likely dietary exposure to cyanogenic glycosides that would result. Both deterministic and probabilistic assessments were conducted.

The deterministic assessment was conducted using 97.5th percentile consumption data and several concentrations in ready-to-eat cassava chips/crisps: the maximum HCN concentration of 145 mg/kg from recent survey data, the mean

Table 9. Estimated acute dietary exposure of the Australian population to total HCN from ready-to-eat cassava chips/crisps at the 97.5th percentile of consumption based on different HCN concentrations (deterministic assessment)

Population group	97.5th percentile consumption (g/kg bw per day)[a]	Dietary exposure to HCN (µg/kg bw per day) at different HCN concentrations			
		10 mg/kg	25 mg/kg	63 mg/kg	145 mg/kg
2+ years	3.1	31	78	195	450
2–4 years	7.2	72	180	454	1044
5–12 years	3.4	34	85	214	493
13–18 years	3.1	31	78	195	450
19+ years	2.0	20	50	126	290

[a] Mean body weights: 2+ years = 67 kg; 2–4 years = 17 kg; 5–12 years = 32 kg; 13–18 years = 62 kg; 19+ years = 74 kg.
Source: FSANZ (2008b)

concentration of 63 mg/kg from the same survey data and two theoretical MLs of 25 mg/kg and 10 mg/kg (equivalent to the Codex ML in cassava flour). The results are shown in Table 9. Children 2–4 years of age have the highest HCN dietary exposure on a body weight basis for all concentrations assessed. At the maximum concentration, dietary exposures ranged from 1044 µg/kg bw per day for children 2–4 years of age to 290 µg/kg bw per day for adults aged 19 years and above. The results show that if all samples were effectively processed during production and an ML of 10 mg/kg was met, estimated dietary exposures would be about 15 times lower than exposures based on maximum concentrations.

For the probabilistic assessment of dietary exposure to HCN from ready-to-eat cassava chips/crisps, a number of scenarios were conducted. The first one was based on the distribution of actual concentration data; the others were based on two simulated distributions (with two sub-scenarios each), based on proposed MLs for HCN of 10 mg/kg and 25 mg/kg. This assessment was for children 2–4 years of age only. Each scenario was run with 10 000 iterations and used the distribution of food consumption data, with all crisps and salty snacks used as a proxy for cassava chips/crisps from the 24-hour recall from the 1995 National Nutrition Survey. Results are shown in Table 10. Based on the actual survey data, mean dietary exposures to HCN are 120 µg/kg bw per day, and high-percentile dietary exposures are 430 µg/kg bw per day. Based on the two scenarios using different proposed MLs, dietary exposures were about half the dietary exposures based on the distribution of survey data for the proposed ML of 25 mg/kg and about 4–6 times lower than the survey data based on the proposed ML of 10 mg/kg.

Acute dietary exposures in Australia based on recent unpublished concentration data from New Zealand submitted to the Committee for a range of foods have also been estimated. Individual dietary records from a 24-hour recall were used to derive 97.5th percentile consumption figures. For the 2007 Australian children's

Table 10. Probabilistic assessment of acute dietary exposure to total HCN for Australian children 2–4 years of age under different scenarios

	Dietary exposure (µg/kg bw per day)				
	Survey data	Proposed ML 10 ± 5 mg/kg	Proposed ML 10 ± 10 mg/kg	Proposed ML 25 ± 10 mg/kg	Proposed ML 25 ± 20 mg/kg
Mean	120	20	20	50	60
97.5th percentile	430	70	100	170	220

Source: FSANZ (2008b)

nutrition survey, which included two 24-hour recalls, only the first 24-hour recall was used to derive the 97.5th percentile consumption figures. If there were not enough consumers of a food to derive a robust 97.5th percentile consumption figure (i.e. less than 39), such as when the dietary survey does not effectively pick up many consumers of occasionally consumed foods, a 95th or 90th percentile consumption amount was derived to represent a high consumer, as they require fewer consumers to derive a robust consumption amount for that percentile (19 and 9 consumers, respectively). Foods with fewer than nine consumers were not included in the assessment. For foods with fewer than 39 consumers where a 95th or 90th percentile consumption figure was used for the dietary exposure estimates, the results may be different from those estimated had a higher number of consumers been available to derive a robust 97.5th percentile consumption figure normally used for acute dietary exposure assessments.The maximum concentration from the small number of samples analysed was used, as there were not enough samples for each food to derive a reliable 97.5th percentile concentration.

Table 11 shows the estimated acute dietary exposures to HCN for a range of foods. The highest acute dietary exposures for children 2–16 years of age were from apple juice, bread containing linseed, cassava and lima beans. For adults 17 years of age and above, the highest acute dietary exposures were from lima beans, prune juice, apple juice, bamboo shoots, cassava, spinach and vine leaves (canned and stuffed). Many beverages are associated with high acute dietary exposures. The HCN concentration for these beverages is low; however, the consumption of these foods is high (e.g. 660 ml of apple juice for adults and 590 ml for children at the 97.5th percentile), resulting in a high level of dietary exposure.

(ii) Chronic dietary exposure estimates

Chronic dietary exposure to HCN was also estimated for Australian adults and children (Table 12). These dietary exposures were based on recent unpublished analytical data in New Zealand (for foods shown in Table 11) and survey data from Australia for ready-to-eat cassava chips/crisps (FSANZ, 2008b). Mean concentrations of HCN for each food were derived from the analytical survey data; the lower-bound mean was calculated by assigning “not detected” results a concentration of zero, and the upper-bound mean was calculated by assigning “not detected” results a concentration equal to the LOD. Distributions of food consumption by individuals from national nutrition survey dietary records were

Table 11. Estimated acute dietary exposures to total HCN for Australian children (2–16 years of age) and adults (17 years and above) for a range of foods

Food name	Children 2–16 years			Adults 17+ years		
	Consumption level used	Dietary exposure to HCN		Consumption level used	Dietary exposure to HCN	
		µg/day	µg/kg bw per day[a]		µg/day	µg/kg bw per day[a]
Almonds	97.5th percentile	215	6	97.5th percentile	450	10
Apple juice	97.5th percentile	4240	110	97.5th percentile	3600	50
Apple sauce	NC	—	—	95th percentile	550	10
Apricots, canned	97.5th percentile	430	11	97.5th percentile	920	10
Bamboo shoots	97.5th percentile	540	14	97.5th percentile	3800	50
Bread containing linseed	97.5th percentile	2170	57	NC	—	—
Butter beans	NA	—	—	NC	—	—
Cassava	97.5th percentile	1850	50	90th percentile	1600	20
Cherry brandy	97.5th percentile	170	4	97.5th percentile	1000	10
Lima beans	90th percentile	1170	31	97.5th percentile	4500	60
Linseed	97.5th percentile	250	7	NC	—	—
Passionfruit	97.5th percentile	300	8	97.5th percentile	360	5
Prune juice	NA	—	—	90th percentile	4300	60
Pumpkin seed	95th percentile	30	1	NC	—	—
Spinach	97.5th percentile	525	14	97.5th percentile	1500	20
Sunflower seed	97.5th percentile	70	2	97.5th percentile	80	1
Vine leaves, stuffed, canned	NA	—	—	90th percentile	1300	20

NA, not assessed due to too few consumers; NC, not consumed

[a] Mean body weights: children 2–16 years = 38 kg; adults 17+ years = 74 kg.

used (two 24-hour recalls for children aged 2–16 years and one 24-hour recall for adults 17 years and above). Mean and 90th percentile dietary exposures were derived from the distribution of estimated exposures. No conversion of cyanogenic glycosides in the body was taken into account in the estimates.

Estimated mean dietary exposures for children were between 18 and 27 µg/kg bw per day, and 90th percentile dietary exposures were between 50 and 71 µg/kg bw per day, based on lower-bound and upper-bound concentrations, respectively.

Table 12. Estimated mean and 90th percentile chronic dietary exposure for consumers of total HCN from a range of foods for Australian adults and children

Age group	Consumers as a % of total respondents[a]		Scenario[c]	Estimated dietary exposure to HCN, µg/day (µg/kg bw per day)[d]			
				Mean		90th percentile	
	Lower bound[b]	Upper bound[b]		Lower bound	Upper bound	Lower bound	Upper bound
Children 2–16 years	88	89	Ready-to-eat cassava chips/ crisps, mean 63 mg HCN/kg	580 (18)	830 (27)	1600 (50)	2100 (71)
			Ready-to-eat cassava chips/ crisps, 10 mg HCN/kg (ML)	170 (10)	430 (10)	400 (10)	1100 (40)
Adults 17+ years	33	36	Ready-to-eat cassava chips/ crisps, mean 63 mg HCN/kg	550 (8)	720 (10)	1800 (27)	2500 (33)
			Ready-to-eat cassava chips/ crisps, 10 mg HCN/kg (ML)	140 (2)	340 (10)	400 (10)	1000 (10)

[a] Number of respondents: children 2–16 years = 4487; adults 17+ years = 11 129.
[b] Lower bound where not detected results = 0, and upper bound where not detected results = LOD.
[c] The estimates of dietary exposure are based on a range of foods. Only the HCN concentration in ready-to-eat cassava chips/crisps changes for each scenario.
[d] Mean body weights: children 2–16 years = 38 kg; adults 17+ years = 74 kg.

For adults, estimated mean dietary exposures were between 8 and 10 µg/kg bw per day, and 90th percentile dietary exposures were between 27 and 33 µg/kg bw per day. The foods that were the major contributors (>5%) to estimated dietary exposures were ready-to-eat cassava chips/crisps (89% adults, 84% children) and apple juice (6% adults, 9% children). All other foods contributed less than 5%.

A second assessment was conducted with ready-to-eat cassava chips/ crisps assigned a concentration at the ML for HCN in Australia and New Zealand of 10 mg/kg. This could be assumed to be a proxy for effective processing methods being employed for this food. Dietary exposures based on the ML for ready-to-eat cassava chips/crisps are about 2–4 times lower than those based on the mean HCN level from analytical survey data (Table 12). This shows that effective processing would result in lower dietary exposures to HCN. The foods that were the major contributors (>5%) to estimated dietary exposures changed when ready-to-eat cassava chips/crisps were assumed to contain HCN at the ML. The ready-to-eat cassava chips/crisps were still the highest contributor (57% adults, 46% children),

followed by apple juice (23% adults, 30% children), cassava (a high contributor only for children, at 7%), almonds (adults only, 10%), linseed (children only, 8%) and bread containing linseed (children only, 5%).

(b) Democratic Republic of the Congo

Dietary exposures to HCN have been estimated for the Democratic Republic of the Congo, based on concentration data from two markets (Ngudi, Kuo & Lambein, 2002). This would be an estimate of usual (chronic) dietary exposure, as it is based on an estimated daily consumption of cossettes (cassava roots that have been soaked and fermented for 3 days, then sun dried).

The dietary exposures are based on a mean concentration (of three samples) of 2.77 mg of HCN equivalents per kilogram dry weight for the Matete market and 1.58 mg of HCN equivalents per kilogram dry weight for the Livulu market. Dietary exposures on a body weight basis were not provided; therefore, the standard internationally used body weight for an adult of 60 kg was used, WHO child growth standards were used to determine mean body weights for children (WHO Multicentre Growth Reference Study Group, 2006) and dietary exposures on a body weight basis were calculated. If actual body weights in these population groups are lower than those used as a result of undernutrition, exposure on a body weight basis would be slightly higher than estimated here.

Estimated mean dietary exposures to HCN are shown in Table 13. For children 1–3 years of age, dietary exposures were 400 and 700 µg/day (or 30 and 60 µg/kg bw per day, respectively). For children 7–9 years of age, dietary exposures were 600 and 1100 µg/day (or 20 and 40 µg/kg bw per day, respectively). For adult females, dietary exposures were 600 and 1100 µg/day (or 10 and 18 µg/kg bw per day, respectively), and for adult males, they were 800 and 1500 µg/day (or 13 and 25 µg/kg bw per day, respectively).

The reported dietary exposures did not include an estimate for high consumers. There is a generally accepted equation that the mean multiplied by 2.5 equates to a 95th percentile (WHO, 1985a). Based on this, estimated dietary exposures to HCN for high consumers would be between 50 and 150 µg of HCN per kilogram body weight for children (1–9 years) and between 25 and 63 µg/kg bw for adults.

(c) Europe

Although not technically “national” assessments, some estimates of dietary exposure have been produced for Europe, some of which were based on combined national consumption data.

Dietary exposure to total HCN has been estimated for European adults 15 years of age and older from food and alcoholic beverages combined. Dietary exposures were approximately 1.6 µg/kg bw per day for consumers with mean levels of consumption and 1.6–1.7 µg/kg bw per day for consumers with high levels of consumption (95th percentile consumption of spirits or fruit brandy), assuming a 60 kg person (EFSA, 2007b). Consumption data for alcoholic beverages came from per capita consumption data from 25 European Union member states.

Table 13. Estimated mean dietary exposure to total HCN from consumption of cossettes for adults and children based on concentration data from two markets in the Democratic Republic of the Congo

Population group	Daily energy intake (kcal)[a]	60% of daily energy provided by cossettes (kcal)	Cossette consumption[b] (g/day)	Estimated mean dietary exposure, µg HCN equivalents/day (µg/kg bw per day[c])	
				Matete market samples	Livulu market samples
Children 1–3 years	1360	816	241	700 (60)	400 (30)
Children 7–9 years	2190	1314	389	1100 (40)	600 (20)
Adult females (moderately active)	2200	1320	390	1100 (18)	600 (10)
Adult males (moderately active)	3000	1800	532	1500 (25)	800 (13)

[a] WHO/FAO/United Nations University energy and protein requirements (WHO, 1985b). 1 kcal = 4.187 MJ.
[b] Based on 338 kcal/100 g cassava.
[c] Based on 60 kg bw for adults, 11.7 kg bw for children 1–3 years, 25.3 kg bw for children 7–9 years.
Source: Adapted from Ngudi, Kudo & Lambein (2002)

Concentration data were those submitted to EFSA for that specific assessment. The dietary exposure from food was the mean dietary exposure to total HCN for Norway of 1.4 µg/kg bw per day from EFSA (2004) (reviewed below). The main contributor to total HCN exposure in average consumers was food products, with alcoholic beverages contributing only minor amounts.

An acute dietary exposure to HCN from alcohol was also calculated. It was based on 95th percentile consumption and concentration data. (This scenario could also be used to represent a brand-loyal consumer for chronic exposures.) This resulted in a dietary exposure of 4.7 µg/kg bw from spirits and food and 24 µg/kg bw from brandy and food, for a 60 kg person. It was concluded by EFSA (2007b) that levels of HCN found in most alcoholic beverages do not pose a risk of acute toxicity, but that measures should be introduced to reduce concentrations of HCN in certain types of beverages because it is a precursor to ethyl carbamate.

Cassava flour is used as a staple food mainly outside Europe. EFSA (2004) noted that consumption of 200 g of flour per person at the ML for HCN of 10 mg/kg would result in an estimated dietary exposure to HCN of 30 µg/kg bw for a 60 kg adult. It was concluded that, based on the view of the Committee in its previous evaluation (Annex 1, reference *101*), such a dietary exposure would not be associated with acute toxicity.

EFSA (2004) also reported a dietary exposure for marzipan, which is made from almonds. The highest level of HCN found in retail samples of marzipan was 20 mg/kg. Based on an estimated consumption of 100 g in one sitting, the dietary exposure would be 2 mg HCN (or 30 µg/kg bw, based on a 60 kg person).

Table 14. Consumption of cassava and bamboo shoots by adults and children in New Zealand from national nutrition survey data

Food	Population group	Number of consumers	Consumers as a % of total respondents[a]	Mean consumption, consumers only (g/day)[b]
Cassava	Children 5–14 years	590	18.0	3
	Adults 15+ years	22	0.5	121
Bamboo shoots	Children 5–14 years	39	1.2	14
	Adults 15+ years	14	0.3	10

[a] Total number of respondents: adults, 1997 National Nutrition Survey = 4636; children, 2002 National Children's Nutrition Survey = 3275.

[b] Based on individual dietary records from one 24-hour recall. Includes where these foods are used as ingredients in mixed foods or dishes.

(d) New Zealand

Consumption data for both cassava and bamboo shoots in New Zealand were submitted to the Committee (Table 14). The proportion of consumers and amounts consumed are low. A single 24-hour recall was used to derive these data (1997 National Nutrition Survey for adults and 2002 National Children's Nutrition Survey). Cassava consumption is largely confined to the Polynesian population (e.g. all consumers among New Zealand adults were Pacific Islanders aged 48–80 years). The higher proportion of cassava consumers for children could be due to a number of factors, including that the survey oversampled Maori and Pacific Islanders, no population weightings were included in the assessment, there is a higher level of detail in the foods consumed for the children's survey, and more detailed recipes for mixed foods were used to capture consumption of core ingredients from all sources in the diet.

(i) Acute dietary exposure estimates

Acute dietary exposure to total HCN was estimated for New Zealand adults from ready-to-eat cassava chips/crisps. The same method was used as for Australia, outlined above. Consumption data for individuals of salty snacks from the 1997 National Nutrition Survey were used in the absence of specific data for ready-to-eat cassava chips/crisps. Several concentrations of HCN were used (maximum of 145 mg/kg from survey data, mean of 63 mg/kg from survey data and two proposed MLs of 25 mg/kg and 10 mg/kg).

The results are given in Table 15. Estimated dietary exposure to total HCN at the 97.5th percentile consumption and maximum concentration were 363 µg/kg bw per day for adults aged 19 years and over. The results show that if all samples were effectively processed during production and an ML of 10 mg/kg was met, estimated dietary exposures would be about 15 times lower than exposures based on the maximum survey concentration.

Table 15. Estimated acute dietary exposure to total HCN from ready-to-eat cassava chips/crisps for New Zealand adults at the 97.5th percentile of consumption based on different HCN concentrations (deterministic assessment)

Population group	97.5th percentile consumption (g/kg bw per day)[a]	Dietary exposure to HCN (µg/kg bw per day) at different HCN concentrations			
		10 mg/kg	25 mg/kg	63 mg/kg	145 mg/kg
15+ years	2.5	25	63	158	363
15–18 years	3.2	32	80	202	464
19+ years	2.5	25	63	158	363

[a] Mean body weights: 15+ years = 71 kg; 15–18 years = 65 kg; 19+ years = 71 kg.
Source: FSANZ (2008b)

Acute dietary exposure estimates based on recent unpublished concentration data from New Zealand submitted to the Committee for a range of foods have also been conducted. Depending on the number of consumers for each commodity, a 97.5th, 95th or 90th percentile consumption amount was derived to represent a consumer with high consumption. The maximum concentration from the small number of samples analysed was used.

Table 16 shows the estimated acute dietary exposures to total HCN for a range of foods. The highest acute dietary exposures for children 5–14 years of age were from apple juice, bread containing linseed, bamboo shoots, taro leaves and lima beans. For adults 15 years of age and older, the highest acute dietary exposures were from cassava, apple juice, canned apricots and apple sauce. Many beverages are associated with a high acute dietary exposure. The HCN concentration for these beverages is low; however, the consumption of these foods is high (e.g. 1000 ml of apple juice for adults and 780 ml for children at the 97.5th percentile), resulting in a high level of dietary exposure.

(ii) Chronic dietary exposure estimates

Chronic dietary exposure to total HCN was also estimated for New Zealand adults and children (Table 17). These dietary exposures were based on recent unpublished analytical data in New Zealand (for foods shown in Table 16) and survey data from Australia for ready-to-eat cassava chips/crisps (FSANZ, 2008b). Distributions of food consumption by individuals from national nutrition survey dietary records (from 24-hour recalls) were used. Mean and 90th percentile dietary exposures were derived from the distribution of estimated dietary exposures. No conversion of cyanogenic glycosides in the body was taken into account in the estimates. Estimated mean dietary exposures for children were between 32 and 36 µg/kg bw per day, and 90th percentile dietary exposures were between 78 and 86 µg/kg bw per day. For adults, estimated mean dietary exposures were between 16 and 15 µg/kg bw per day, and 90th percentile dietary exposures were between 50 and 42 µg/kg bw per day. The foods that were the major contributors (>5%) to estimated dietary exposures were cassava chips/crisps (91% adults, 93% children). All other foods contributed less than 5%.

Table 16. Estimated acute dietary exposures to total HCN for children aged 5–14 years and adults aged 15 years and above in New Zealand

Food name	Children 5–14 years			Adults 15+ years		
	Consumption level used	Dietary exposure to HCN		Consumption level used	Dietary exposure to HCN	
		µg/day	µg/kg bw per day[a]		µg/day	µg/kg bw per day[a]
Almonds	97.5th percentile	260	10	97.5th percentile	380	10
Apple juice	97.5th percentile	4200	100	97.5th percentile	5 700	80
Apple sauce	90th percentile	550	10	97.5th percentile	1 100	20
Apricots, canned	97.5th percentile	460	10	97.5th percentile	1 130	20
Bamboo shoots	97.5th percentile	1500	40	90th percentile	520	10
Bread containing linseed	95th percentile	2600	60	NC	—	—
Butter beans	NC	—	—	NC	—	—
Cassava	97.5th percentile	730	20	95th percentile	21 300	300
Cherry brandy	90th percentile	20	1	97.5th percentile	480	10
Lima beans	95th percentile	1100	30	90th percentile	310	4
Linseed	97.5th percentile	590	10	NC	—	—
Linseed oil	NC	—	—	NC	—	—
Linseed, sunflower seed and almond (mix)	NC	—	—	NC	—	—
Marzipan	NC	—	—	NC	—	—
Passionfruit	97.5th percentile	320	10	97.5th percentile	240	3
Pumpkin seed	90th percentile	30	1	NC	—	—
Spinach	97.5th percentile	590	10	97.5th percentile	870	10
Sunflower seed	97.5th percentile	20	1	97.5th percentile	60	1
Taro leaves only	95th percentile	1200	30	NA	—	—

NA, not assessed due to too few consumers; NC, not consumed

[a] Mean body weights: children 5–14 years = 40 kg; adults 15+ years = 71 kg.

A second assessment was conducted with ready-to-eat cassava chips/crisps at the ML in Australia and New Zealand of 10 mg/kg. This could be assumed to be a proxy for effective processing methods being employed for this food. Dietary exposures based on the ML for ready-to-eat cassava chips/crisps

Table 17. Estimated mean and 90th percentile chronic dietary exposure for consumers to total HCN from a range of foods for the New Zealand population groups of adults and children

Age group	Consumers as a % of total respondents[a]		Scenario[b]	Estimated dietary exposure to HCN, µg/day (µg/kg bw per day)[c]			
				Mean		90th percentile	
	Lower bound	Upper bound		Lower bound	Upper bound	Lower bound	Upper bound
Children 5–14 years	71	73	Ready-to-eat cassava chips/ crisps, mean 63 mg HCN/kg	1140 (32)	1270 (36)	2700 (78)	3000 (86)
			Ready-to-eat cassava chips/ crisps, 10 mg HCN/kg (ML)	250 (10)	400 (10)	500 (20)	1000 (30)
Adults 15+ years	21	29	Ready-to-eat cassava chips/ crisps, mean 63 mg HCN/kg	1140 (16)	1040 (15)	3200 (50)	3200 (42)
			Ready-to-eat cassava chips/ crisps, 10 mg HCN/kg (ML)	270 (4)	400 (11)	600 (10)	1100 (20)

[a] Number of respondents: children 5–14 years = 3275; adults 15+ years = 4636.
[b] The estimates of dietary exposure are based on a range of foods. Only the concentration for ready-to-eat cassava chips/crisps changes for each scenario.
[c] Mean body weights: children 5–14 years = 40 kg; adults 15+ years = 71 kg.

are about 2–5 times lower than those based on the mean from analytical survey data. This shows that effective processing would result in lower levels of dietary exposure to HCN (Table 17). The foods that were the major contributors (>5%) to estimated dietary exposures changed when ready-to-eat cassava chips/crisps were assumed to contain the ML. The ready-to-eat cassava chips/crisps were still the highest contributor (61% adults, 61% children), followed by cassava (16%, both adults and children) and apple juice (16%, both adults and children). All other foods contributed less than 5%.

(e) Norway

Maximum limits of HCN exist in Europe for foods to which flavourings or other food ingredients with flavouring properties have been added. Dietary exposure to total HCN from these sources has been estimated for Norway. Norwegian adults (16–79 years) had a mean dietary exposure to HCN of 95 µg/person (1.4 µg/kg bw per day) for consumers and a 97.5th percentile dietary exposure of 372 µg/person (5.4 µg/kg bw per day) for consumers (EFSA, 2004). These dietary exposures were based on data from a Norwegian dietary survey (NORKOST 1997) (National Council on Nutrition and Physical Activity, 1999) using concentrations as per proposed Council of Europe limits. It is assumed that this is a chronic dietary exposure assessment, as it discusses the inclusion of multiple foods. However, no further details were presented in relation to the type of dietary survey or the specific foods included. It is also possible that this estimate may not reflect actual

dietary exposures, as it is based on MLs. It was concluded by EFSA (2004) that the estimated exposure to cyanide from flavouring ingredients (97.5th percentile) is unlikely to give rise to acute toxicity.

(f) United Kingdom

Bitter apricot kernels have been marketed as a health food in the United Kingdom. They contain high levels of the cyanogenic glycoside amygdalin. Consumption advice provided on packets of bitter apricot kernels suggest eating 5 kernels in an hour and no more than 10 kernels in a day. However, other information on the Internet suggests that cancer sufferers should consume 5 kernels per hour up to 6–10 times per day (i.e. a total of 30–50 kernels per day). The mean cyanide concentration of the kernels recently on sale was 1450 mg/kg (0.5 mg/kernel). Acute dietary exposures were estimated. Consumption of 5 kernels in an hour would lead to a dietary exposure to cyanide of 2500 µg (42 µg/kg bw). For 10 kernels per day, cyanide dietary exposure would be 5000 µg (83 µg/kg bw per day). Thirty kernels per day would result in a dietary exposure to cyanide of 15 000 µg (250 µg/kg bw), and 50 kernels per day would result in a dietary exposure to cyanide of 25 000 µg (420 µg/kg bw per day) (Committee on Toxicity, 2006).

Mean and high (97.5th percentile) daily dietary exposures to HCN for United Kingdom adults (16–64 years) from its presence in ingredients used in flavours or flavouring ingredients have been estimated as 46 µg/person (0.8 µg/kg bw per day) and 214 µg/person (3.6 µg/kg bw per day), respectively (EFSA, 2004). These dietary exposures have been calculated based on data from the Dietary and Nutritional Survey of British Adults (a 7-day weighted record of food consumption from 2197 respondents) and maximum levels in foods according to the proposed Council of Europe limits. It is assumed that this is a chronic dietary exposure assessment, as it discusses the inclusion of multiple foods. However, no further details were presented in relation to the specific foods included or methods used. It is also possible that this estimate may not reflect actual dietary exposures, as it is based on MLs. It was concluded that the current exposure to cyanide from flavouring ingredients (97.5th percentile) is unlikely to give rise to acute toxicity.

9.3.2 International estimates of dietary exposure

In order to obtain a global perspective and to permit regional comparisons of the potential exposure to a contaminant, the Committee usually estimates chronic dietary exposure for contaminants using the GEMS/Food consumption cluster diets (WHO, 2006) and concentrations of the contaminant in relevant foods as obtained for the assessment in question. The available occurrence data for cyanogenic glycosides were deemed to be inappropriate for use in determining international estimates of chronic dietary exposure to HCN in combination with the GEMS/Food consumption cluster diets. There were insufficient data for some foods or no concentration data for prepared or processed foods, which are more reflective of the concentrations in foods as consumed. In addition, cyanogenic glycosides occur in many processed foods, and consumption data for many of these foods are not included in the cluster diets. Therefore, no international estimates of chronic dietary exposure were prepared. The same issues with the occurrence data did not

allow acute dietary exposures to be estimated using the WHO large portion (97.5th percentile) food consumption data.

9.3.3 Impact of maximum levels for foods on dietary exposure to cyanogenic glycosides

MLs of HCN have been established by Codex and in a number of different countries for foods including sweet cassava, cassava flour, gari and ready-to-eat cassava chips/crisps and in many foods containing flavouring agents. There are no new proposed MLs or proposed revisions to existing MLs that require evaluation in this assessment; however, the impact of the existing MLs was evaluated where data were available.

In addition, some of the dietary exposure estimates in previous parts of this report have estimated levels of dietary exposure when MLs in foods are considered. For Australia and New Zealand, estimated chronic and acute dietary exposures were calculated for a range of foods, including ready-to-eat cassava chips/crisps, based on concentrations from an analytical survey (mean HCN concentration of 63 mg/kg or maximum HCN concentration of 145 mg/kg) and based on the Australia/New Zealand ML of 10 mg/kg (see above). Chronic dietary exposures based on the ML for ready-to-eat cassava chips/crisps are about 2–5 times lower than those based on the mean concentration from analytical survey data (depending on the population groups assessed). Acute dietary exposures based on the ML for cassava chips/crisps were about 4–14 times lower than those based on analytical survey data.

Mean consumption values are available from the GEMS/Food consumption cluster diets for cassava and cassava flour. Therefore, dietary exposures to HCN were estimated based on existing MLs for these foods. There were no consumption data for gari, so estimates based on the Codex ML for HCN of 2 mg/kg were not conducted. The Codex ML of 50 mg/kg for HCN is based on the definition for the maximum concentration for sweet cassava. There is also a Codex ML for HCN in cassava flour of 10 mg/kg; therefore, dietary exposures were estimated based on this level. There was no consumption of cassava or cassava flour for clusters B, C, D, E and F; therefore, these were not included in the evaluation. The estimated dietary exposures are shown in Table 18. Estimated dietary exposures to HCN for consumption of sweet cassava based on the ML range between 1 and 235 µg/kg bw per day, depending on the cluster. For cassava flour, estimated dietary exposures to HCN based on an ML of 10 mg/kg ranged from less than 0.1 to 14 µg/kg bw per day.

This information shows that application of the MLs results in lower estimated dietary exposures, particularly when involving food in trade and assuming that monitoring and enforcement take place.

Maximum acute dietary exposures, if regulatory limits were enforced, were also estimated. These calculations were based on MLs using consumption information from the WHO large portion data set (Table 19). As the consumption data are based on raw commodities, there were no consumption amounts for processed commodities of interest to this assessment (e.g. cassava flour or gari).

Table 18. Estimated dietary exposure to HCN for cassava and cassava flour based on the GEMS/Food consumption cluster diets and Codex maximum levels

(a) Consumption clusters A, G, H and I

Food	ML (mg HCN/kg)	A			G			H			I		
		Consumption (g/day)	Dietary exposure		Consumption (g/day)	Dietary exposure		Consumption (g/day)	Dietary exposure		Consumption (g/day)	Dietary exposure	
			µg/day	µg/kg bw per day[a]		µg/day	µg/kg bw per day[a]		µg/day	µg/kg bw per day[a]		µg/day	µg/kg bw per day[a]
Cassava	50	242.8	12 140	202	15.6	780	13	23.9	1195	20	171.3	8565	143
Cassava flour	10	14.3	143	2	1.6	16	0.3	2.5	25	0.4	0.3	3	0.1

(b) Consumption clusters J, K, L and M

Food	ML (mg HCN/kg)	J			K			L			M		
		Consumption (g/day)	Dietary exposure		Consumption (g/day)	Dietary exposure		Consumption (g/day)	Dietary exposure		Consumption (g/day)	Dietary exposure	
			µg/day	µg/kg bw per day[a]		µg/day	µg/kg bw per day[a]		µg/day	µg/kg bw per day[a]		µg/day	µg/kg bw per day[a]
Cassava	50	282.2	14 110	235	57.7	2885	48	20.0	1000	17	0.8	40	0.7
Cassava flour	10	82.7	827	14	10.2	102	2	0	—	—	0.1	1	<0.1

[a] Based on a body weight of 60 kg.

Table 19. Estimated acute dietary exposures to total HCN based on WHO large portion data using maximum concentration found in survey data and some Codex maximum levels

Codex classification code	Commodity name	97.5th percentile consumption (g/kg bw per day)[a]		HCN concentration (mg/kg)	Concentration reference	Dietary exposure to HCN (µg/kg bw per day)	
		General population	Children ≤6 years			General population	Children ≤6 years
VR463	Cassava (assume sweet)	3.01	6.63	50	ML definition for sweet cassava	150	330
VR463	Cassava (assume as flour)	3.01	6.63	10	Codex ML flour	30	70

[a] WHO (2008).

It was therefore assumed that the WHO 97.5th percentile consumption value for cassava is the same as that for cassava flour, which is a conservative assumption. For cassava, estimated dietary exposures to HCN based on the ML of 50 mg/kg were 150 µg/kg bw for the general population and 330 µg/kg bw for children. Estimated dietary exposures to HCN for cassava flour based on the ML of 10 mg/kg would be 30 µg/kg bw for the general population and 70 µg/kg bw for children.

The ML for sweet cassava is for the raw product. If the starting level of HCN in the raw sweet cassava is 50 mg/kg, the minimum effective processing, which reduces the concentration by 70%, would result in an HCN concentration of 15 mg/kg, and the most effective processing, which reduces the concentration by 97%, would give an HCN concentration of 2 mg/kg.

9.4 Other routes of exposure

Exposure to cyanide can come from many sources, including food, water, air, dust and cigarette smoke and occupational exposure (industrial plants and cassava processing plants). Occupational exposure occurs mainly via inhalation, with dermal absorption to a lesser degree (e.g. from airborne particles during fumigation) (IPCS, 2004).

Exposure to HCN from air for non-urban non-smokers has been estimated to be 3.8 µg/day (based on an HCN concentration of 190 ng/m^3 and average air intake of 20 m^3) (IPCS, 2004). This would be equivalent to 0.06 µg/kg bw per day for a 60 kg adult. Concentrations of HCN in the air of industrial factories (a plating facility of an electrical and electronic company) between 0.07 and 4.3 mg/m^3 have been reported (IPCS, 2004). If the total amount of air inhaled per day is 20 m^3 and one third of a day is spent at work (approximately 7 m^3 inhaled at work), then exposure to HCN for industrial workers would be between 0.5 and 30 mg/day (8–500 µg/kg bw per day for a 60 kg adult) (assuming that HCN at a concentration of 190 ng/m^3 in air is inhaled during non-work time). This is up to 130 times more than exposures for non-industrially exposed persons. The air near large cassava processing plants in Nigeria contained cyanide at levels between 20 and 46 mg/m^3. For people living near these plants and inhaling this air all the time, intakes of cyanide would be between 400 and 920 mg/day (based on 20 m^3 inhaled per day, or 7–15 mg/kg bw per day, based on a 60 kg adult).

Some estimates from occupational exposure have been documented. Occupationally exposed industrial workers in India had higher mean urinary thiocyanate levels (316 µmol/l) than non-occupationally exposed individuals (90 µmol/l) (IPCS, 2004). Okafor, Okorowkwo & Maduagwu (2002) studied occupational exposure of cassava processors and exposure of cassava consumers in Nigeria. Mean urinary thiocyanate levels of workers were 2.2 and 2.6 times higher than those of frequent and infrequent consumers of cassava, respectively (details in Table 3).

Cyanides can be found in drinking-water, primarily as a consequence of industrial contamination (WHO, 2011b). Exposure to cyanide from drinking-water has been estimated to be 0.4–0.7 µg (based on water consumption of 2 litres and levels of cyanogen chloride in water equivalent to cyanide levels of 0.19–0.34 µg/l)

(IPCS, 2004). This would equate to a cyanide exposure of 0.006–0.012 µg/kg bw per day for a 60 kg adult.

No estimates of exposure to cyanides from cigarette smoke were found; however, thiocyanate levels in the blood of smokers have been reported to be about 3 times those of non-smokers (FAO/WHO, 1965). Mean urinary thiocyanate levels have been reported to be 146 ± 9.9 µmol/l in adult smokers (>10 cigarettes/day) (Mlingi et al.,1998). It was also reported from Cuba that smoking 20 cigarettes per day results in an exposure to cyanide at least 5 times that associated with consuming 1 kg of boiled cassava (Hernandez et al., 1995).

Inhalation of the air in non-industrial areas would result in exposures to HCN that are lower than those from food; however, inhalation exposure could be much higher than dietary exposures in industrialized areas. Exposure from water is also estimated to be lower than exposure from food.

9.5 Limitations and uncertainties associated with the exposure estimates

There are some limitations and uncertainties associated with the estimates of dietary exposure to cyanogenic glycosides. These relate to the assessment inputs, such as consumption and concentration data, the resulting dietary exposure estimates, biomarkers of exposure and assessing all sources of exposure.

Only a small number of concentration data are available for foods other than cassava; this makes it difficult to estimate both acute and chronic dietary exposures, particularly for countries where cassava is not a staple food.

For acute dietary exposure assessments, there were often no or not enough individual data points to allow a robust 97.5th percentile concentration to be derived and used for the dietary exposure estimates. Mean, other high-percentile (e.g. 95th) or maximum levels were therefore often used. Maximum concentrations may be quite high if from a positively skewed distribution, which is usually the case for contaminant concentration data.

Some analytical data have non-detectable or non-quantifiable concentrations; therefore, there is a level of uncertainty about what the actual level is in the food. Assumptions are then made about the numerical concentration to assign to these results for deriving mean concentrations for use in chronic dietary exposure estimates (e.g. zero or a level equal to the LOD for the analytical method). This results in some level of uncertainty in the estimates of chronic dietary exposure.

Concentrations of cyanogenic glycosides in foods vary with climatic conditions and season. They are also highly variable depending on processing and food preparation. Also, the food supply and standards for processed foods are regularly changing; therefore, so are cyanogenic glycoside concentrations in foods. In Australia and New Zealand, for example, estimated dietary exposures were based on survey data for ready-to-eat cassava chips/crisps that were collected before the ML for this food was put in place in 2009; thus, using the older survey data for dietary exposure estimates could lead to estimates that are higher than those at present if the crisps now have lower concentrations that meet the ML. Many of these limitations associated with the occurrence data make it difficult to determine representative concentrations to use for estimating dietary exposures.

There are some limitations associated with the food consumption data used for the dietary exposure estimates. There can be limited consumption of certain foods in some dietary surveys. This could be due to the age of the survey and certain foods not being on the market at the time of the survey, such as ready-to-eat cassava chips/crisps, linseed, sunflower seed and almond mix and linseed-containing breads for Australian and New Zealand adults (1995 and 1997 surveys, respectively). Assumptions then need to be made about the consumption of these foods, such as using all potato crisps and salty snacks as a proxy for ready-to-eat cassava chips/crisps for Australia and New Zealand.

Limited consumption data may also be a result of the low proportion of people who consume the food within a population or the commodity being infrequently consumed. This may mean that there are insufficient consumers to derive a robust 97.5th percentile consumption figure for use in acute dietary exposure estimates. A 7-day weighted record of food consumption such as that used in the United Kingdom provides more reliable and robust consumption data for use in dietary exposure estimates and is especially good for estimating chronic dietary exposure. Some countries may not have national dietary surveys. In these cases, consumption estimates based on energy requirements and energy content of foods have been used, such as the estimates conducted in the Democratic Republic of the Congo (Ngudi, Kuo & Lambein, 2002); however, this would not produce estimates of dietary exposure as reliable as those derived from survey data.

There are some limitations associated with estimates of dietary exposure to HCN (only from MLs in this assessment) based on the GEMS/Food consumption cluster diets. The diets are based on food balance sheet data, which concern amounts of food available for consumption. These tend to be higher than actual amounts consumed, as they do not take into consideration losses of food due to waste or variation of consumption within a population. This may result in the dietary exposures being overestimated. Despite these limitations, the consumption cluster diets do provide relative dietary exposure estimates between the clusters; therefore, they can show what areas of the world are likely to have higher dietary exposures than others.

Estimates of dietary exposure reviewed have been for total HCN. This is because most of the concentration data are presented in this form. However, some of the occurrence data outlined above demonstrate that foods can contain cyanogenic glycosides (e.g. linamarin in cassava), cyanohydrin or HCN. These levels will vary depending on the level of processing of the food (the more processing, the more likely that there will be less cyanogenic glycoside and more HCN). The form that remains in the food is also reliant on the pH of the food and the temperature used for processing the food. The form that is in the food as consumed will influence the dietary exposure to cyanide. For example, if linamarin is in the food, some of it is excreted intact, resulting in less dietary exposure.

It is difficult to compare dietary exposure estimates for total HCN, as each one has been conducted in slightly different ways, which could be due to the population groups assessed, the foods included, the type of consumption data used, the assumptions made or how the dietary exposures are reported (e.g. reported as all respondents [per capita] compared with consumers only). Dietary exposure

to cyanogenic glycosides is highly dependent on the type and effectiveness of processing or preparation of the foods containing cyanogenic glycosides, so the estimates may only be a reflection of the dietary exposure based on the foods for which concentration data are available.

It is difficult to take into account from estimates of dietary exposure to HCN the actual level of exposure to cyanide or the cyanogenic glycosides that this relates to because of many influencing factors, such as intraindividual and interindividual variation in detoxification in the body, how much of the ingested amount is absorbed or excreted intact, the nutritional status of those exposed, dietary intake of sulfur-containing amino acids, social situations, etc.

Dietary exposures to cyanogenic glycosides have been considered primarily from food. No estimates included dietary exposure to cyanide from drinking-water, although dietary exposures to cyanide from drinking-water have been evaluated separately. Exposures to cyanide from the air and occupational exposure have also been estimated separately. It is difficult to estimate a total exposure from all these sources combined, particularly when they are all estimated from different population groups from different regions of the world with totally different levels of exposure and calculation methods used. Also, non-dietary exposures from occupations and cigarette smoking can result in high levels of exposure, but only for the relevant population groups.

There are limitations associated with biomarkers of exposure as outlined above, but the main one of relevance to the dietary exposure estimates is that they do not quantitatively link estimates of cyanide exposure to numerical levels consumed from the diet. This is difficult to determine due to the body's metabolism, the nutritional status of the exposed, etc. One of the key uses of this information is therefore in identifying population groups with higher levels of exposure.

Overall, it is difficult to determine representative levels of dietary exposure for cyanogenic glycosides for risk characterization purposes because of the different ways in which each assessment has been undertaken, including the different types and numbers of foods, different dietary surveys used, dietary exposures for population groups that have vastly different levels of exposure due to staple foods consumed and different calculation methods.

9.6 Summary of the dietary exposure assessment

Foods containing cyanogenic glycosides are consumed in varying amounts depending on the region of the world. Cassava in particular is highly consumed in parts of Africa, for example. Acute (short-term) and chronic (long-term) estimates of dietary exposure have been evaluated. A summary of the national estimated dietary exposures is shown in Table 20. Chronic estimates of dietary exposure have been summarized separately for two population groups: those reliant on cassava as a staple food (such as in Africa) and those who are not, because these different dietary patterns will lead to different levels of dietary exposure to cyanogenic glycosides.

There is a large range in the estimates of both acute and chronic dietary exposures. This is due to two main factors: first, because of the variation in dietary

Table 20. Summary of national dietary exposure estimates for total HCN

Type of dietary exposure assessment	Population group	No. of dietary exposure estimates	Range of reported means (µg/kg bw per day)	Range of reported high percentiles (µg/kg bw per day)
Acute	Children	2	NA	1–1044
	Adults	4	NA	<1–430
Chronic	Children (cassava not a staple)	2	18–36	50–86[a]
	Children (cassava a staple)	1	20–60	50–150[b]
	Adults (cassava not a staple)	5	<1–16	2–50[c]
	Adults (cassava a staple)	1	10–25	25–63[b]

NA, mean dietary exposures are not applicable for acute dietary exposure estimates

[a] 90th percentile.

[b] 95th percentile.

[c] 90th, 95th or 97.5th percentile, depending on the assessment.

exposures between countries reliant on cassava as a staple and those that are not, but second, and more so, because of the level of uncertainty in the concentration data used for the assessments, particularly for foods other than cassava.

There is a broad range of acute dietary exposure estimates, as they depend on the consumption amount of the food, which is highly variable for foods containing cyanogenic glycosides, and the concentration of cyanogenic glycosides used, which varied between mean and maximum concentrations, depending on the assessment.

Estimated chronic dietary exposures to HCN from flavouring ingredients are shown to be low (as indicated by European estimates of dietary exposure). Estimated dietary exposures from cassava can be high, as cassava is the primary contributor to dietary exposures to cyanogenic glycosides in tropical regions. For countries where cassava is not consumed as a staple food, estimated chronic dietary exposures can be high if they are influenced by particular foods, such as ready-to-eat cassava chips as the major contributor for estimates for Australia and New Zealand.

MLs for total HCN have been established by Codex and in a number of countries for foods such as sweet cassava, cassava flour, gari, ready-to-eat cassava chips/crisps and many foods containing flavouring agents. Estimates of dietary exposure were calculated for Australia and New Zealand, for which analytical survey data for ready-to-eat cassava chips (collected before the ML was established) were substituted with the ML of 10 mg/kg. Chronic dietary exposure estimates are about 2–5 times lower than estimated dietary exposures based on mean survey values for cassava chips. Acute dietary exposures were about 4–14

times lower than estimated dietary exposures based on survey data for cassava chips.

Acute dietary exposures, using the Codex ML for sweet cassava of 50 mg/kg, based on WHO large portion consumption data were between 150 and 330 µg/kg bw per day. Assuming that the consumption of cassava flour is equivalent to that of cassava, estimated exposure to HCN based on the Codex ML of 10 mg/kg would be 30–70 µg/kg bw per day. Chronic dietary exposures to HCN for sweet cassava using the Codex ML of 50 mg/kg, based on consumption amounts from the GEMS/Food consumption cluster diets, were 1–235 µg/kg bw per day for the clusters assessed. For cassava flour, based on the Codex ML for HCN of 10 mg/kg, exposures ranged from less than 0.1 to 14 µg/kg bw per day.

The limitations associated with the dietary exposure estimates need to be taken into consideration in relation to the risk characterization undertaken for this evaluation.

Biomarkers of exposure are useful for determining population groups with higher levels of exposure to cyanogenic glycosides. These groups include populations that have experienced toxicological effects of cyanide exposure (e.g. with konzo), consumers with high cassava consumption, consumers of insufficiently processed cassava, people who are cigarette smokers, those who are occupationally exposed (e.g. cassava processors) and those with iodine deficiency disorders and poor nutritional status.

10. DOSE–RESPONSE ANALYSIS AND ESTIMATION OF CARCINOGENIC/TOXIC RISK

10.1 Identification of key data for risk assessment

10.1.1 Pivotal data from biochemical and toxicological studies

For the risk assessment of cyanogenic glycosides, the most critical effect observed in the toxicological database was skeletal defects in hamsters administered linamarin from the Frakes, Sharma & Willhite (1985) developmental toxicity study.

For the risk assessment of cyanide, critical effects observed in the toxicological database were reproductive effects in male mice and rats from the NTP (1993) study, and rats appeared to be the more sensitive species.

In the majority of species tested to date with relatively low repeated doses of cyanide compounds, reduced organ weights, altered sperm parameters, increased thyroid weight and altered thyroid hormone levels have been reported. However, comparisons of these studies (Jackson, 1988; Kamalu, 1993; Soto-Blanco, Maiorka & Górniak, 2002a,b; Soto-Blanco & Górniak, 2003, 2004; Manzano et al., 2007) are complicated by differences in dosing procedures, use of animals with compromised health or nutritional status and insufficient reporting of incidences, severity and statistical significance of observed effects. Also, many studies used only one or two dose levels and are therefore not suitable for dose–response analysis.

In the 13-week NTP (1993) study, groups of rats and mice (both sexes, 10 animals per dose group) were administered six increasing concentrations of sodium cyanide over a 300-fold range in the drinking-water. Treatment-related mortality was not observed, but statistically significant male reproductive effects in rats and mice that increased in severity in a dose-dependent manner were reported. Observed effects included decreased absolute cauda and whole epididymis weights, decreased testes weight and decreased testicular spermatid count, among other altered sperm parameters; rats were more sensitive to these effects than mice. From this study, statistically significantly decreased absolute and relative cauda epididymis weights were observed in male rats at doses greater than or equal to 1.4 mg/kg bw per day.

10.1.2 Pivotal data from human clinical/epidemiological studies

Although toxicological effects in humans have been induced by cyanogenic glycosides following exposure through various routes, the available data from epidemiological investigations and studies that include biomarkers of exposure or effect were not considered suitable for assessing overall risk.

10.2 General modelling considerations

Dose–response modelling of toxicological data was used to determine the basis for hazard characterization. In general, dose–response modelling of toxicological data is used to determine a point of departure for further risk assessment. Dose–response data were used to derive the 95% lower confidence limit of the benchmark dose (BMDL) for developmental end-points in hamsters and reproductive end-points in male rats.

10.2.1 Selection of data for estimates of BMDs and BMDLs

The Frakes, Sharma & Willhite (1985) developmental toxicity study in hamsters was considered to be the pivotal study for risk assessment because the exposure to linamarin was by the oral route and occurred during a sensitive life stage. The most prominent dose–response effect of linamarin in this developmental toxicity study in hamsters was an increased number of fetal skeletal defects. Data were not reported by the study authors for individual litters; therefore, quantal data on a fetal basis were considered, as no significant differences between control and treated groups were observed with respect to the total numbers of litters, implantations or live fetuses, fetal body weight and maternal weight gain.

The NTP (1993) bioassay in the rat was considered to be the pivotal study for risk assessment of cyanide, because it employed low-dose exposures to sodium cyanide in drinking-water over 13 weeks. The most prominent dose–response effect of sodium cyanide was a decrease in the absolute weight of the cauda subsection of the epididymis for male rats at all dosages. Relative cauda epididymis weight was statistically significantly decreased in all treated groups compared with controls, but it was not dose dependent. Other end-points from this study were also considered (i.e. terminal body weight, epididymis weight, testis weight, testicular spermatid count and spermatozoal motility) for benchmark dose (BMD) modelling

(data not shown); however, the resulting BMDLs generated were greater than those estimated for decreased absolute cauda epididymis weight or were not amenable to modelling. In support of this data set, decreased cauda epididymis weight was observed in mice administered significantly higher oral doses of sodium cyanide.

As additional indications of cauda epididymis perturbation such as sperm maturation, fertilization capacity and cauda epididymis sperm count were not evaluated in this study, the biological significance of the observed weight decrease is unknown.

10.2.2 Measure of exposure

A single linamarin dose of 70, 100, 120 or 140 mg/kg bw dissolved in 0.9% sodium chloride was administered by oral gavage to pregnant hamsters on day 8 of gestation (Frakes, Sharma & Willhite, 1985).

Male F344 rats were administered sodium cyanide in drinking-water for 13 weeks at concentrations of 0, 3, 10, 30, 100 or 300 mg/l, which were calculated to be equal to cyanide doses of 0, 0.16, 0.48, 1.4, 4.5 and 12.5 mg/kg bw per day (NTP, 1993).

10.2.3 Selection of mathematical model

(a) Modelling procedure for continuous data

BMD modelling was conducted using the United States Environmental Protection Agency's BMD software (BMDS version 2.1.2), with all available continuous models (i.e. exponential, Hill, linear, polynomial, power). Benchmark responses (BMRs) of one standard deviation (1SD) of the control mean or 5% and 10% extra risk were modelled for comparison purposes. An adequate fit was judged based on the goodness-of-fit *P*-value ($P > 0.05$), scaled residual closest to the BMR and visual inspection of the model fit. In addition to the three criteria for judging adequate model fit, whether the variance needed to be modelled and, if so, how it was modelled also determined final use of the model results. If a homogenous variance model was recommended based on statistics provided from the BMD model runs, the final BMD results would be estimated from a homogenous variance model. If the test for homogenous variance was negative ($P < 0.05$), the model was run again while applying the power model integrated into the BMDS to account for non-homogenous variance (known as the non-homogenous model). If the non-homogenous variance model did not provide an adequate fit to the variance data, the data set would be considered unsuitable for BMD modelling. Models that passed the goodness-of-fit test ($P > 0.05$) were considered to be acceptable; from these models, the lowest BMDL was selected.

(b) Modelling procedure for dichotomous data

BMD modelling for dichotomous data was conducted with the USEPA's BMD software (BMDS version 2.1.2) using all dichotomous models (i.e. gamma, multistage, logistic, log-logistic, probit, log-probit, Weibull, quantal linear and dichotomous Hill) available within the software, with a default BMR of 10%, 5%

Table 21. Incidence of skeletal defects from Frakes, Sharma & Willhite (1985)

Linamarin dose (mg/kg bw)	Number of hamsters dosed	Number of litters	Number of fetuses examined	Number of skeletal defects
0	11	11	67	1
70	11	8	55	0
100	10	9	56	5
120	11	9	54	10
140	13	10	63	14

Table 22. Dose–response modelling of skeletal defects from Frakes, Sharma & Willhite (1985)

Model	*P*-value	AIC	BMD/BMDL for linamarin (mg/kg bw)					
			BMD_{10}	$BMDL_{10}$	BMD_{05}	$BMDL_{05}$	BMD_{01}	$BMDL_{01}$
Gamma[a]	0.303	172.02	110.12	97.46	94.26	75.79	69.00	42.93
Logistic	0.2313	172.378	107.06	95.53	83.42	68.17	38.03	22.37
Log-logistic[b]	0.2726	172.398	110.421	96.96	93.61	74.14	64.99	39.23
Log-probit[b]	0.3388	171.703	109.473	97.05	94.14	76.82	70.93	47.74
Multistage 2[c]	0.1877	174.548	99.93	85.26	69.72	53.12	30.86	13.74
Multistage 3[c]	0.3884	171.657	105.87	94.22	83.29	67.46	48.37	19.96
Probit	0.1496	173.192	103.61	91.21	78.11	63.0	34.59	19.69
Weibull[a]	0.2527	172.617	110.86	97.01	93.39	73.16	63.33	36.99

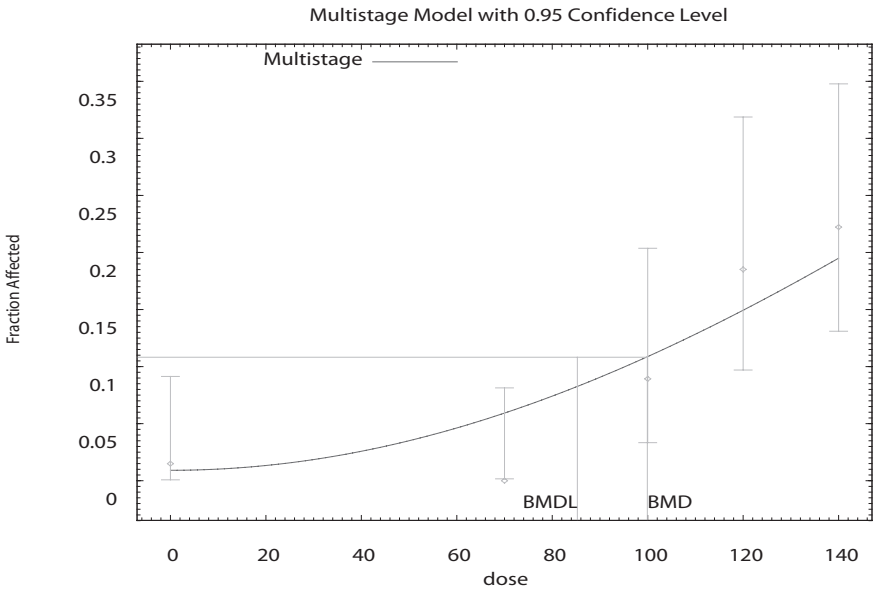
[a] Power parameter ≥1.
[b] Slope ≥1.
[c] Beta ≥1.

and 1% extra risk. An adequate fit for each model was judged based on the goodness-of-fit *P*-value ($P > 0.05$), scaled residual closest to the BMR and visual inspection of the model fit. The Akaike's information criterion (AIC) is calculated by $-2L + 2p$, where L is the log-likelihood at the maximum likelihood estimates for the parameters and p is the number of model parameters estimated. The AIC can be used to compare different types of models that use a similar fitting method, as do all dichotomous and continuous model types within BMDS. Among the models providing adequate data fit, the lowest BMDL was selected if the BMDLs estimated from different models varied greater than 3-fold. Models that passed the goodness-of-fit test ($P > 0.05$) were considered to be acceptable.

10.3 BMD analyses

All available quantal models in the BMDS (version 2.1.2) were fit to the skeletal defects data for hamsters given a single dose of linamarin by gavage in

Figure 4. Multistage 2 (degree of polynomial = 2) model of skeletal defects from Frakes, Sharma & Willhite (1985)



the Frakes, Sharma & Willhite (1985) study (Table 21). For comparison purposes, BMRs of 10%, 5% and 1% were used for the BMD modelling. As assessed by the chi-squared goodness-of-fit statistic, all models except for the multistage (degree of polynomial = 1) and the quantal linear provided adequate fit to the data (Table 22).

As the AICs and BMDs estimated by the acceptable models are similar, the lowest BMDL may be selected as a point of departure. The estimated BMD_{10} and $BMDL_{10}$ (the BMD and BMDL for a 10% response) for skeletal defects are 99.93 and 85.26 mg/kg bw per day for linamarin, respectively. Figure 4 shows the observed incidence of fetal skeletal defects fitted to the multistage 2 model (degree of polynomial = 2).

All available continuous models in the BMDS (version 2.1.2) were fit to the reproductive parameters (cauda epididymis weight, testicular spermatid count, epididymis weight, testes weight) reported for male rats in the NTP (1993) study. For comparison purposes, BMRs corresponding to a change in the mean equal to one standard deviation of the control group and a 5% or 10% change in the control mean were employed for the BMD modelling (Table 23). None of the continuous models for relative cauda epididymis weight and testicular spermatid count passed the goodness-of-fit test for acceptance. Absolute reproductive organ weights (Table 24) were selected for modelling instead of relative organ weights, as body weight decreases were considered minimal (6% in the high-dose group) and there is evidence that absolute testes, cauda and whole epididymis weights are not altered in rodents in spite of body weight reductions of up to 30% (Chapin et al., 1993). Furthermore, decreases in epididymis weight would likely precede substantial

Table 23. Dose–response modelling of cauda epididymis weight for rats

Model	*P*-value	AIC	BMD/BMDL for cyanide (mg/kg bw per day)					
			BMD_{10}	$BMDL_{10}$	BMD_{05}	$BMDL_{05}$	BMD_{1SD}	$BMDL_{1SD}$
Exponential 2[a]	0.091	–312.91	11.43	7.78	5.57	3.79	8.10	5.31
Exponential 3[a]	0.091	–312.91	11.43	7.78	5.57	3.79	8.10	5.31
Exponential 4[a]	0.207	–314.11	4.33	0.017	1.22	0.005	1.87	0.008
Exponential 5[a]	0.207	–314.11	4.33	0.022	1.22	0.006	1.87	0.007
Hill[b]	0.350	–314.83	4.41	0.611	0.90	0.03	1.48	0.071
Polynomial 1E (linear)[c]	0.084	–312.75	11.64	8.12	5.82	4.06	8.40	5.64
Polynomial 2E[d]	0.107	–313.10	5.99	3.09	2.39	1.37	3.51	1.90
Power[a]	0.084	–312.75	11.64	8.12	5.82	4.06	8.40	5.64

[a] Power parameter ≥1.
[b] Slope ≥1.
[c] Restricted non-positive polynomial coefficient.
[d] Unrestricted polynomial coefficient.

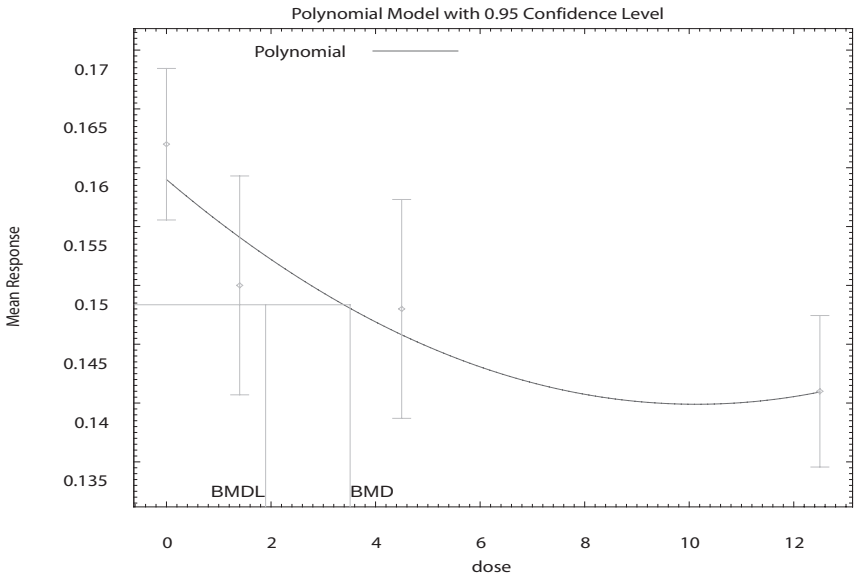
Table 24. Absolute cauda epididymis weight in rats from NTP (1993)

Cyanide dose (mg/kg bw per day)	*n*	Cauda epididymis weight (g) (mean ± standard deviation)
0	10	0.162 ± 0.009
1.4	10	0.150 ± 0.013
4.5	10	0.148 ± 0.013
12.5	10	0.141 ± 0.009

decrements in sperm parameters and fertility in this species. Although the Hill and exponential (4 and 5) models meet the established criteria for model acceptance, the BMD and BMDL estimates are highly model dependent given the relatively wide ratio between these values and consequently were excluded from the evaluation (Barlow et al., 2006; Benford et al., 2010). Also, as the Hill model requires the estimation of four parameters (i.e. intercept, *v*, *n* and *k*), which necessitates having a minimum of five dose groups in order to have adequate degrees of freedom for testing model fit, the results were not considered.

As the AICs and BMDs estimated for the acceptable models are similar, the lowest BMD may be selected as the point of departure. The estimated BMD_{1SD} and $BMDL_{1SD}$ (the BMD and BMDL for a BMR of one standard deviation of the control mean) for cyanide for decreased absolute cauda epididymis weight are 3.5 and 1.9 mg/kg bw per day, respectively. Figure 5 shows the dose–response relationship for absolute cauda epididymis weight for the secondary polynomial model (unrestricted polynomial coefficient) for a one standard deviation BMR response.

Figure 5. Secondary polynomial model of rat absolute cauda epididymis weight from NTP (1993)



10.4 Conclusions from dose–response analysis

In the dose–response analysis, statistical models were fit to experimental data that were considered relevant for further consideration. Those resulting in acceptable fits based on biological and statistical considerations were selected to derive the BMDs and BMDLs. This procedure results in a range of BMD and BMDL values for each end-point considered.

The range of $BMDL_{10}$s for linamarin estimated based on the incidence of fetal skeletal defects in hamster is 85.3–97.5 mg/kg bw. The more conservative lower end of this range of values is recommended for use in the evaluation.

The range of $BMDL_{1SD}$s for cyanide estimated based on the decrease in absolute cauda epididymis weight for rats is 1.9–5.6 mg/kg bw per day. The lower end of this range of values is recommended as a point of departure in the evaluation.

11. COMMENTS

11.1 Absorption, distribution, metabolism and excretion

The biotransformation of cyanogenic glycosides involves two main steps: 1) cleavage of the carbohydrate moiety by β-glucosidases and 2) subsequent enzymatic or spontaneous dissociation of the cyanohydrin to the corresponding aldehyde or ketone and HCN.

Following oral administration, a proportion of ingested cyanogenic glycosides is absorbed and excreted intact in the urine. In limited experiments with human volunteers exposed to cyanogenic glycosides—either linamarin in cassava food products or oral doses of purified amygdalin—between 8% and 32% of the cyanogenic glycoside dose was absorbed and excreted unmetabolized in urine. The residual fraction of unabsorbed cyanogenic glycosides can be enzymatically converted by microorganisms in the gastrointestinal tract to cyanohydrins, following cleavage of the carbohydrate moiety, and then eventually to the corresponding aldehyde and/or ketone and HCN. In experimental animals pretreated with antibiotics, a substantial reduction or elimination of the conversion of cyanogenic glycoside to cyanide is observed. In addition, supplementation of cassava-based experimental diets with D,L-methionine overcomes a dietary deficiency of sulfur-containing amino acids and provides an available source of labile sulfur for cyanide detoxification.

HCN is readily absorbed after oral administration and rapidly distributed in the body through the blood. The concentration of cyanide is higher in erythrocytes than in plasma, as it is known to bind with iron in both methaemoglobin and haemoglobin present in erythrocytes. At the physiological pH of the stomach, cyanide will predominantly form HCN, which can rapidly penetrate mucous and cell membranes. However, after oral exposure, HCN is subjected to extensive presystemic metabolism by the liver.

Cyanide binds to and inactivates several enzymes, particularly those containing iron in the ferric (Fe^{3+}) state and cobalt. Examples include cytochrome *c* oxidase, catalase, peroxidase, xanthine oxidase and succinic dehydrogenase. Cyanide produces histotoxic anoxia by binding to the active site of cytochrome *c* oxidase, the terminal protein in the electron transport chain located within the mitochondrial membrane.

Cyanide is detoxified in the liver by conversion to thiocyanate by the intramitochondrial enzyme rhodanese (thiosulfate–cyanide sulfur transferase). Rhodanese catalyses the transfer of sulfur from a donor to cyanide to form thiocyanate. The availability of sulfur-containing donor molecules is the rate-limiting factor in the detoxification of cyanide. Several polymorphisms in rhodanese have been identified in human populations, although only a minimal effect on cyanide detoxification has been detected. Approximately 80% of cyanide is estimated to be detoxified by conversion to thiocyanate, which undergoes renal clearance with a half-life of approximately 2.7 days. Cyanide can also be detoxified by direct chemical combination with sulfur-containing amino acids (e.g. L-cysteine and L-methionine) or by conjugation with hydroxocobalamin to form cyanocobalamin. Detoxification of cyanide is therefore affected by the presence of nutritional factors, such as sulfur-containing amino acids and vitamin B12.

11.2 Toxicological data

Acute toxicity and mortality induced by various cyanogenic glycosides in experimental animals are directly related to, and influenced by, factors associated with the release and detoxification of HCN. As with the previous evaluation, the Committee considered it appropriate to evaluate toxicological data related to

both cyanogenic glycosides and HCN. LD_{50}s of various cyanogenic glycosides (linamarin, prunasin, amygdalin) have been reported to range from 450 to 880 mg/kg bw in experimental animals. Symptoms associated with acute toxicity are largely similar to those observed with cyanide exposure (metabolic acidosis, decreased cytochrome oxidase activities, atrial fibrillation and decreased respiratory rates).

The majority of animal experiments with cyanogenic glycosides involve only limited numbers of test animals consuming diets containing some fraction of cassava or cassava extract. Effects observed include body weight decrease, impaired glucose tolerance and nonspecific histopathological changes in liver, kidney, thyroid and the central nervous system, including cellular vacuolization, degeneration and necrosis. These effects are consistent with a combination of protein deficiency and cyanide toxicity.

Experimental animals given diets consisting of at least 50% fresh cassava (8–10 mg/kg as HCN equivalents) exhibit significant decreases in body weight within 3 months and signs of decreased motor coordination related to protein malnutrition. Exposure of pregnant hamsters to protein-deficient diets supplemented with cassava meal resulted in delayed fetal ossification and an increased percentage of pups per litter with a reduced body weight. Direct gavage dosing of maternal animals at a critical period of organogenesis (GD 8) with linamarin or amygdalin produced a dose-dependent increase in fetal skeletal defects and malformations but no significant change in associated litter parameters, including number of litters with prenatal deaths, number of live fetuses per litter and fetal body weight. Simultaneous exposure to amygdalin and a cyanide antagonist, thiosulfate, significantly reduced the incidence of congenital malformations.

Oral LD_{50} values for cyanide in experimental animals average between 2 and 4 mg/kg bw. Signs of acute toxicity generally occur within minutes of dosing. A similar range of oral human lethal doses has also been reported (0.5–3.5 mg/kg bw).

In various experimental animal species (rats, pigs, goats) exposed to HCN, potassium cyanide or sodium cyanide, qualitative histopathological changes of the central nervous system and thyroid have been reported at continual doses of 0.1–30 mg/kg bw per day (exposure via diet or drinking-water). Thyroid effects noted included increased vacuolization and weight changes, sometimes reported in the absence of thyroid hormone alterations, whereas central nervous system effects reported included neuron loss in the hippocampus, loss of cerebellar white matter and delayed maturation and migration of external granular layer neurons in the cerebellum. However, comparisons of these studies are complicated by differences in dosing procedures, use of animals with compromised health or nutritional status and insufficient reporting of incidences, severity and statistical significance of observed effects.

Subchronic repeated administration of sodium cyanide via drinking-water provided evidence of effects on the male reproductive system in both rats and mice, including decreased epididymis and cauda epididymis weights, decreased testicular sperm counts and decreased sperm motility.

Experimental data for multigenerational reproductive toxicity, chronic toxicity and carcinogenicity are not available for cyanide. Overall, in vitro and in vivo genotoxicity assays for cyanide have been negative.

11.3 Observations in humans

Accidental poisoning due to cyanide has been reported as a result of ingestion of foods containing cyanogenic glycosides. Whole blood concentrations of HCN in children with non-fatal episodes of poisoning were 0.32 and 0.56 mg/l, whereas the blood levels in two fatal cases were 0.85 and 1.35 mg/l. The cyanide blood level of an adult with acute cyanide toxicity caused by ingestion of apricot kernels was 1.1 mg/l 5 hours after ingestion. The estimated cyanide exposure was 0.73 mg/kg bw. Cancer patients treated with 0.5 g of laetrile (amygdalin) 3 times per day (25 mg/kg bw per day as amygdalin) had widely varying levels of cyanide in blood, with 5th and 95th percentiles of approximately 0.1 and 2.3 mg/l, respectively; two thirds of these patients had blood concentrations below 1 mg/l.

Long-term consumption of cassava containing high levels of cyanogenic glycosides, usually when cassava constitutes the main source of calories, has been associated with neurological diseases, mainly involving endemic spastic paraparesis (konzo) and tropical ataxic neuropathy. In areas with low iodine intake, development of hypothyroidism and goitre, sometimes accompanied by these neurological diseases, has also been linked to cassava consumption.

Epidemic outbreaks of konzo have been reported in the Democratic Republic of the Congo, Mozambique, the United Republic of Tanzania and the Central African Republic, with potential causal links with high consumption of inadequately processed cassava or reliance on cassava as a diet staple. The epidemiological association between cassava consumption and konzo is considered to be consistent; however, definitive interpretation is complicated by underlying protein and nutrient deficiencies in the populations in question.

Tropical ataxic neuropathy is used to describe several neurological syndromes attributed to toxico-nutritional causes. The syndromes grouped as tropical ataxic neuropathy can differ widely in clinical presentation and characteristics. The main clinical features include sore tongue, angular stomatitis, skin desquamations, optical atrophy, neurosensory deafness and sensory gait ataxia. Tropical ataxic neuropathy has occurred mainly in Africa, particularly Nigeria, but it has also been recently reported in India. Although it has been found associated with consumption of a diet dominated by processed cassava foods, the results of recent studies are not consistent. The confounding effect of nutritional deficiencies cannot be ruled out.

Iodine deficiency disorders, which are endemic in certain African countries, comprise various significant adverse health effects, including goitre, hypothyroidism, mental retardation, cretinism, and increased neonatal and infant mortality. High serum levels of thiocyanate, which acts as an iodide transport inhibitor, can occur after exposure to cyanide from cassava consumption. Cassava consumption has been associated with goitre in both children and mothers, but only when urinary iodine levels were below 10 µg/dl, indicating iodine deficiency, or when intake of iodine was consistently below 100 µg/day.

Acute cyanide toxicity may be caused by ingestion of inadequately processed cassava or other foods containing cyanogens; nevertheless, the available studies do not provide a link between a defined intake and the occurrence of adverse effects due to acute cyanide exposure. There is a consistent association between konzo epidemics and diets consisting mainly of inadequately processed cassava, which involves high cyanide exposure. The Committee, however, concluded that the current data from epidemiological studies were insufficient to form the basis for establishing a health-based guidance value.

11.4 Analytical methods

The compounds of health concern found in food derived from cyanogenic plants are 1) the cyanogenic glycosides initially present in the plants used as food, 2) the cyanogenic glycosides and cyanohydrins that remain in food after processing and food preparation and, ultimately, 3) the HCN and/or cyanide that is present in the food or that could be released from cyanogenic glycosides and cyanohydrins remaining in food when it is consumed. A number of colorimetric and instrumental (GLC and HPLC) methods are available for measuring individual cyanogenic glycosides, cyanohydrins, HCN or cyanide in food.

The term "total HCN" was used by the Committee to describe the total HCN content of all cyanogenic glycosides, cyanohydrins and "free" HCN in a food.

Direct measurement of total HCN by, for example, acid hydrolysis of cyanogenic glycosides present in foods and decomposition of the intermediate cyanohydrins to HCN has the advantages of being applicable to all types of samples and of generating a result directly comparable with Codex MLs (expressed as total milligrams of HCN per kilogram). However, this can lead to an overestimate of dietary exposure, as not all potential HCN of cyanogenic glycosides is likely to be available in vivo. Analysis of individual cyanogenic glycosides or cyanohydrins by GLC or HPLC, especially if using mass spectrometric detection, has the advantage that both chemical characterization and quantification are achieved.

11.5 Levels and patterns of contamination in food commodities

Levels of cyanogenic glycosides, measured as total HCN, in plants used as food or for flavouring can vary greatly, depending on both cultivar and environmental factors. The highest levels reported (expressed as HCN) are associated with bamboo shoots (range 70–8000 mg/kg), bitter almonds (300–4700 mg/kg), lima beans (not detected to 3120 mg/kg) and bitter apricot kernels (90–4000 mg/kg). In regard to cassava tubers, two categories are recognized based on Codex definitions: sweet cassava, less than 50 mg/kg as total HCN; and bitter cassava, greater than 50 mg/kg of cyanides, expressed as HCN (fresh weight basis). The range of total HCN concentrations reported, however, in nominally sweet cassava by various authors is 1–1064 mg/kg as HCN. The range of total HCN concentrations reported in bitter cassava is 15–1120 mg/kg. Bitter almonds and bitter apricot kernels are among the nuts and fruits used to make cyanogenic glycoside–containing flavours for use in products such as marzipan and liqueurs. From a flavouring point of view, the most important are those liberating benzaldehyde as a flavouring compound—namely,

amygdalin, sambunigrin and prunasin. Drought conditions can lead to significantly higher levels of cyanogenic glycosides, even in sweet cassava tubers.

Information about the sampling procedures and number of samples for each food was not provided in detail for many of the papers evaluated.

11.6 Prevention and control

The Codex Alimentarius Commission has developed and published standards for sweet cassava, edible cassava flour and gari. The key aspects of these standards are as follows: sweet cassava, less than 50 mg/kg of "hydrogen cyanide"; edible cassava flour, "total hydrocyanic acid" must not exceed 10 mg/kg; and gari, "total hydrocyanic acid" must not exceed 2 mg/kg as "free" HCN.

Selection of low-cyanogen cultivars and transgenic methods of reducing cyanogenic glycosides in foods such as cassava have been reported in the literature.

11.7 Effects of processing

If the initial level of cyanogenic glycosides in the food is high, commonly used food processing methods may not ensure reduction in the level of total HCN to below Codex MLs. For example, using the least efficient method for making cassava flour from peeled tubers (sun drying), up to 30% of the total HCN present in the cassava has been shown to remain in the flour. The total HCN level in the cassava used must therefore be less than 30 mg/kg to ensure achieving the ML of 10 mg/kg as HCN in cassava flour.

When the most efficient processing method (crushing and sun drying) is used, approximately 3% of the original total HCN remains in the flour. This means that processing cassava containing greater than 330 mg/kg as total HCN is unlikely to achieve the Codex ML of 10 mg/kg as total HCN in cassava flour. This can be the case, for example, when very bitter rather than sweet cassava is used in the preparation of flour or during periods of drought, when higher than normal levels of cyanogenic glycosides are found in cassava tubers. Thus, as well as using the most effective methods of food preparation, the selection and use of cultivars low in cyanogenic glycosides are particularly important if Codex MLs are to be met.

Food processing as a means of detoxification also depends on the availability of endogenous glycosidase enzymes in the food being processed. It has been shown, in the case of bamboo shoots, for example, that HCN removal is less effective at temperatures above 100 °C, where the glycosidase enzyme is denatured. In relation to cassava, higher degrees of maceration and high levels of moisture result in improvements in HCN removal.

Another aspect that needs to be considered relates to the stability of cyanohydrins at low pH. Gari, a product obtained from processing cassava tubers, has a pH of 4.1, and the cyanogens remaining in gari comprise up to 85% acetone cyanohydrin and only 15% linamarin, whereas cassava flour, a product with a pH of 6.5, contains mainly linamarin and very little acetone cyanohydrin because, at the higher pH of the flour, the acetone cyanohydrin generated readily decomposes to

HCN and acetone. This has an impact on the relative potential for gari and cassava flour of equal total HCN content to produce HCN in vivo. The acetone cyanohydrin, which is the predominant cyanogen in gari, releases all its HCN in weakly alkaline environments (e.g. in the intestines), whereas less than 50% of linamarin, the predominant cyanogen in cassava flour, is estimated to be converted to cyanide in the body. Thus, the same total HCN values recorded on analysis of gari and cassava flour do not reflect equal toxicities; as a consequence, gari has a Codex ML of 2 mg/kg as HCN, reflected by "free" HCN, whereas the Codex ML for cassava flour is 10 mg/kg as total HCN.

11.8 Dietary exposure assessment

The dietary exposure estimates evaluated included those submitted to the Committee for Australia and New Zealand and other information found in the literature (primarily for Africa and Europe). Both acute and chronic estimates of dietary exposure were considered. The estimated dietary exposures were generally expressed as exposure to total HCN, as this was the form recorded in most of the occurrence or analytical data. However, dietary exposure can be to cyanogenic glycosides, cyanohydrins or HCN, depending on the processing of the food. The use of total HCN for the dietary exposure estimates represents the maximum exposure to cyanide coming from substances derived from cyanogenic glycosides in foods.

11.8.1 National estimates of acute dietary exposure

Acute dietary exposures to total HCN were estimated using mean, maximum or high-percentile (e.g. 95th percentile) concentrations of total HCN from analytical data. Consumption data used for the estimates were amounts per day of individual foods for consumers with maximum or high consumption at the 97.5th, 95th or 90th percentile, where available.

Estimated acute dietary exposures to total HCN for a range of foods from the small number of countries for which information was available ranged between 1 and 1044 µg/kg bw per day, depending on the food and the population groups assessed. Foods leading to the highest estimates were bitter apricot kernels (estimates up to 440 µg/kg bw per day in the United Kingdom), cassava (300 µg/kg bw per day for adults in New Zealand), ready-to-eat cassava chips (up to about 1000 µg/kg bw per day for children and up to 370 µg/kg bw per day for adults in Australia and New Zealand) and apple juice (100–110 µg/kg bw per day for children in Australia and New Zealand).

11.8.2 National estimates of chronic dietary exposure

National estimates of chronic dietary exposure to total HCN were available for Australia, the Democratic Republic of the Congo, Europe, New Zealand, Norway and the United Kingdom. These estimates are based on different food sources, including raw and processed foods and foods in which cyanogenic glycosides occur as a result of flavouring uses.

Estimated chronic dietary exposures to total HCN from the countries for which information was available were between less than 1 and 60 µg/kg bw per day

Table 25. Summary of national estimates of chronic dietary exposure to total HCN

Country or region	Mean dietary exposures (µg/kg bw per day)		High-percentile dietary exposures (µg/kg bw per day)	
	Children	Adults	Children	Adults
Australia[a]	18–27	8–10	50–71	27–33
Democratic Republic of the Congo[b]	20–60	10–25	50–150	25–63
Europe[c]	—	1.6	—	1.7
New Zealand[a]	32–36	15–16	78–86	42–50
Norway[d]	—	1.4	—	5.4
United Kingdom[d]	—	0.8	—	3.6

[a] Includes a range of foods. High percentile is the 90th. Range of results is from lower bound to upper bound.
[b] Included only cassava cossettes. Range is based on analytical data from two different markets. High percentile is the 95th.
[c] Exposures from proposed MLs in foods as flavouring agents. High percentile is the 95th.
[d] Exposures from proposed MLs in foods as flavouring agents. High percentile is the 97.5th.

for consumers with average exposure and between 2 and 150 µg/kg bw per day for consumers with high exposure. The estimates are summarized in Table 25.

11.8.3 International estimates of dietary exposure

The available occurrence data for cyanogenic glycosides were deemed not to be appropriate for use in determining international estimates of dietary exposure to total HCN in combination with the GEMS/Food consumption cluster diets for chronic dietary exposure or with WHO 97.5th percentile large portion data for acute dietary exposure. There were insufficient occurrence data for some foods or no concentration data for prepared or processed foods, which are more reflective of the concentrations in foods as consumed. In addition, cyanogenic glycosides occur in many processed foods, and consumption data for many of these foods are not included in the consumption cluster diets or large portion data. Therefore, no international estimates of chronic or acute dietary exposure were prepared.

11.8.4 Evaluation of existing maximum levels in relation to dietary exposure to cyanogenic glycosides

MLs for total HCN have been established by Codex and in a number of countries for foods including sweet cassava, cassava flour, gari and ready-to-eat cassava chips/crisps and for many foods containing flavouring agents. Estimates of chronic and acute dietary exposure to total HCN were calculated for Australia and New Zealand, for which analytical survey data for ready-to-eat cassava chips (collected before the FSANZ ML was established) were substituted with the ML of 10 mg/kg. This resulted in mean chronic dietary exposures of 10 µg/kg bw per day

for children and 2–11 µg/kg bw per day for adults and 90th percentile exposures of 10–40 µg/kg bw per day for children and 10–12 µg/kg bw per day for adults. These chronic exposure estimates are about 2–5 times lower than estimated dietary exposures based on mean survey values for cassava chips. If all cassava chips were at the ML of 10 mg/kg, the estimated acute dietary exposures to total HCN would be up to a maximum of 100 µg/kg bw per day for children and 25 µg/kg bw per day for adults. These acute estimates are about 4–14 times lower than estimated dietary exposures based on mean survey values for cassava chips.

Acute dietary exposures based on WHO large portion consumption data for sweet cassava using an HCN concentration of 50 mg/kg were 150 µg/kg bw per day for the general population and 330 µg/kg bw per day for children. There was no consumption value for cassava flour in the large portion data set, but if it was assumed that the consumption of cassava flour is equivalent to that of cassava, estimated exposure to HCN based on the Codex ML of 10 mg/kg would be 30 µg/kg bw per day for the general population and 70 µg/kg bw per day for children.

Chronic dietary exposures to HCN from sweet cassava and cassava flour were estimated based on consumption amounts from the GEMS/Food consumption cluster diets and MLs. For sweet cassava at a maximum HCN level of 50 mg/kg (Codex limit for sweet cassava), estimated dietary exposures ranged between 1 and 235 µg/kg bw per day for the clusters assessed. For cassava flour, based on the Codex ML of 10 mg/kg as total HCN, exposures ranged between less than 0.1 and 14 µg/kg bw per day.

11.8.5 Biomarkers of dietary exposure

Biomarkers of exposure were evaluated as they relate to dietary patterns such as the frequency of consumption of cassava and cassava products. No studies were found that quantitatively link measured levels of biomarkers to numerical estimates of dietary exposure. The information evaluated indicated that consumers of cassava have higher urinary thiocyanate levels than those who never consume cassava; weekly consumers had levels between those of daily consumers and non-consumers. Frequent (e.g. twice per day) or high levels of consumption of cassava can result in low levels of urinary thiocyanate (<100 µmol/l) when effective processing reduces the levels of cyanogenic glycosides. Consumption of varieties of cassava with low levels of HCN results in lower levels of urinary thiocyanate and linamarin. Levels of urinary inorganic sulfate are higher for those who consume a broader range of foods in the diet (other than cassava), which include other amino acids (including sulfur-containing amino acids). Biomarker levels tend to be seasonal, with peaks occurring during times of cassava harvest when cassava consumption is higher. Results from a study in human volunteers suggest that individual differences in liberation of cyanide from ingested linamarin are more important for the internal cyanide exposure.

11.9 Dose–response analysis

The Committee recognized that human exposure to HCN from cyanogenic glycosides in food commodities would be from a combination of intact glycoside,

partially degraded glycoside (cyanohydrin moiety) and totally degraded glycoside (residual HCN). The ratio of contributors to total food HCN equivalents will vary depending on the commodity and extent of processing.

Following a review of both the experimental and human data specific to cyanogenic glycosides, the Committee concluded that, although there were relevant studies in experimental animals for establishing an acute reference dose (ARfD[1]) for oral exposure, there were no appropriate studies on which to base a long-term health-based guidance value. However, as the potential toxicity of ingested cyanogenic glycosides is directly related to the in situ generation of HCN, the Committee concluded that experimental animal studies with cyanide compounds could serve as the basis for establishing a provisional maximum tolerable daily intake (PMTDI).

The Frakes, Sharma & Willhite (1985) developmental toxicity study in hamsters was selected for dose–response assessment, as the exposure to linamarin was by the oral route and occurred during a sensitive life stage. Similar developmental effects have also been reported in the same animal model with higher doses of related cyanogenic glycosides (amygdalin and prunasin). The most prominent dose–response effect of linamarin in this developmental toxicity study in hamsters was an increased number of fetal skeletal defects. Data were not reported by the study authors for individual litters; therefore, quantal data on a fetal basis were considered. In the same study, the total number of litters, litters with prenatal deaths, number of live fetuses per litter and fetal body weight were not significantly different between the treated groups and control.

A single linamarin dose of 0, 70, 100, 120 or 140 mg/kg bw was administered by oral gavage to pregnant hamsters on day 8 of gestation, with the fetuses removed on GD 15 and examined for internal and external malformations. BMD modelling for dichotomous data was conducted with the USEPA's BMD software (BMDS version 2.1.2) using all dichotomous models available within the software, with a default BMR of 10% extra risk. All default constraints as set by the program were accepted. Models that passed the goodness-of-fit test ($P > 0.05$) were considered to be acceptable. As the AICs and BMDs estimated by the acceptable models were all similar, the lowest BMDL was selected as a point of departure. The estimated ranges of BMD_{10}s and $BMDL_{10}$s (the BMD and BMDL for a 10% response) for skeletal defects are 100–111 and 85–98 mg/kg bw per day for linamarin, respectively.

Reproductive effects observed in male rats exposed to doses ranging from 1.4 to 12.5 mg/kg bw per day as cyanide over 13 weeks in drinking-water included decreased absolute and relative cauda epididymis weight as well as decreased testis weight and testicular sperm count at the highest dose tested. Although additional measurements directly related to cauda epididymis perturbation, such as sperm maturation, fertilization capacity and cauda epididymis sperm count, were not evaluated in this study, a slight reduction in sperm motility (statistically

[1] An ARfD by definition is an estimate of the "amount of a substance in food and/or drinking water, normally expressed on a body weight basis, that can be ingested in a period of 24 h or less without appreciable health risk to the consumer on the basis of all known facts at the time of the evaluation" (FAO/WHO, 2002).

significant) was also noted at all doses, although it was not dose dependent. In addition, related effects associated with the male reproductive tract, such as reduced epididymal and testis weights and reduced testicular sperm counts, were observed at the highest sodium cyanide dose tested (12.5 mg/kg bw per day as cyanide). Taken in combination, these data are indicative of a toxic effect on the male reproductive system in rats.

All available continuous models in the BMDS (version 2.1.2) were fitted to the reproductive parameters (cauda epididymis weight, testicular spermatid count, epididymis weight, testes weight) reported for male rats in the NTP (1993) study. As the biological significance of the observed degree of change in this end-point was unclear, the BMR of one standard deviation (1SD) change in the control mean was considered to be the most appropriate. None of the continuous models for relative cauda epididymis weight and testicular spermatid count passed the goodness-of-fit test for acceptance ($P > 0.05$). Absolute reproductive organ weights were selected for modelling instead of relative organ weights, as body weight decreases were considered minimal (6% in the high-dose group), and there is evidence that absolute weights of testes, cauda and whole epididymis are not altered in rodents in spite of body weight reductions of up to 30% (Chapin et al., 1993).

The ranges of BMD_{1SD} and $BMDL_{1SD}$ values estimated based on the decrease in absolute cauda epididymis weight for rats are 3.5–8.4 and 1.9–5.6 mg/kg bw per day as cyanide, respectively. The lower end of the $BMDL_{1SD}$ range was selected as a point of departure in the evaluation.

12. EVALUATION

Reports of acute human poisoning associated with the consumption of foods containing cyanogenic glycosides were reviewed. The Committee therefore considered it appropriate to establish an ARfD for cyanogenic glycosides, expressed as cyanide equivalents. In addition, as there are a number of human diseases, specifically konzo, tropical ataxic neuropathy and iodine deficiency disorders, associated with the chronic consumption of underprocessed cassava as a staple food, it was recognized that the derivation of a chronic health-based guidance value would also be relevant.

12.1 Derivation of the ARfD

Following review of a developmental toxicity study with linamarin (Frakes, Sharma & Willhite, 1985), the Committee considered this study as suitable for establishing an ARfD. BMD modelling of the data from this study provided a $BMDL_{10}$ for linamarin of 85 mg/kg bw for increased skeletal defects in developing hamster fetuses following acute exposure of maternal animals. While the study did not use dietary exposure, gavage dosing was considered relevant to establish the ARfD.

Following application of a 100-fold uncertainty factor, the Committee established an ARfD for linamarin of 0.9 mg/kg bw (equivalent to 0.09 mg/kg bw as cyanide). This value was considered, when compared on a cyanide molar basis, to be applicable also to other cyanogenic glycosides. Therefore, the Committee

recommended conversion of the ARfD for linamarin to a cyanide-equivalent dose of 0.09 mg/kg bw. This cyanide-equivalent ARfD applies only to foods containing cyanogenic glycosides as the main source of cyanide.

12.2 Derivation of the PMTDI

In a 13-week NTP (1993) study not previously evaluated by the Committee, in which exposure to sodium cyanide was continuous via drinking-water, a variety of effects related to male reproductive organs were observed—namely, decreased cauda epididymis weights, decreased testis weights and decreased testicular spermatid concentration. Dose–response analysis of continuous data on absolute cauda epididymis weights generated the lowest $BMDL_{1SD}$ of 1.9 mg/kg bw per day. On the basis of this $BMDL_{1SD}$, the Committee established a PMTDI of 20 µg/kg bw by applying a 100-fold uncertainty factor. The Committee decided that it was not necessary to apply an additional uncertainty factor to account for the absence of a long-term study, considering the generally acute nature of cyanide toxicity and the sensitivity of the effect (i.e. the reduction of absolute cauda epididymis weight).

12.3 Comparison of estimated dietary exposures with health-based guidance values and the impact of maximum levels on dietary exposure

Estimated dietary exposures to total available HCN were converted to cyanide equivalents and compared with the health-based guidance values established by the Committee at this meeting.

From the national acute dietary exposure estimates available to the Committee for review, the ARfD of 90 µg/kg bw as cyanide equivalents was exceeded 3-fold for cassava for adults (based on raw samples), less than 2-fold for apple juice for children, between 2- and 5-fold for bitter apricot kernels and up to 10-fold for ready-to-eat cassava chips/crisps, depending on the population group. If ready-to-eat cassava chips contained a level equivalent to the recently established ML in Australia and New Zealand of 10 mg/kg as HCN, there was only a marginal exceedance of the ARfD for children. These results are based on dietary exposure to total HCN, which represents the maximum possible exposure for foods containing cyanogenic glycosides.

Based on national estimates of chronic dietary exposure to total HCN, there is also the potential to exceed the PMTDI of 20 µg/kg bw as cyanide for populations reliant on cassava as a staple food: between 1- and 3-fold in children and between 1- and 2-fold in adults. There is also a potential for those populations not reliant on cassava to exceed the PMTDI: between 1- and 5-fold for children and between 1- and 3-fold for adults. For Australia and New Zealand, ready-to-eat cassava chips were the major contributor to dietary exposure to HCN (84–93%). When the cassava chips contain a level equivalent to the ML of 10 mg/kg as HCN, all mean dietary exposures were below the PMTDI. High-percentile exposures for children were between 1- and 2-fold above the PMTDI. All chronic dietary exposure estimates based on exposures from flavouring agents did not exceed the PMTDI. These results are based on dietary exposure to total HCN, which is a worst-case scenario.

Table 26. Maximum amount of cassava or cassava food products that could be consumed based on different MLs so that the health-based guidance values are not exceeded[a]

Health-based guidance value[b]	ML (mg/kg as HCN)	Maximum amount of cassava or cassava products that can be consumed (g/day)
ARfD	50	110
	10	560
	2	2800
PMTDI	50	25
	10	125
	2	620

[a] Based on a body weight of 60 kg.
[b] ARfD of 90 µg/kg bw per day as cyanide equivalents; PMTDI of 20 µg/kg bw as cyanide.

Application of the ML of 50 mg/kg as HCN for sweet cassava could result in dietary exposures that exceed the ARfD by less than 2-fold for the general population and up to 4-fold for children and exceed the PMTDI by between 2- and 10-fold, depending on the population group assessed. These estimates do not take into consideration any reduction in concentration of total HCN as a result of food preparation or processing. For the ML of 10 mg/kg as HCN for cassava flour, there are no estimates of dietary exposure available that exceed the ARfD or PMTDI. This is supported by the maximum amount of food that can be consumed based on existing Codex MLs before the health-based guidance values would be exceeded (Table 26), which is as low as 25 g/day for cassava for chronic exposure. More detailed estimates of cassava and cassava flour consumption and concentrations in food for cassava-eating communities would help in supporting the conclusion that dietary exposures to total HCN could exceed health-based guidance values.

The ML for sweet cassava is for the raw product. If the starting level of HCN in the raw sweet cassava were 50 mg/kg as HCN, the minimum effective processing would result in a concentration of 15 mg/kg as HCN, and the most effective processing would give an HCN concentration of 2 mg/kg.

12.4 Research needs

Further research is needed to more accurately quantify how nutritional factors ultimately contribute to the human diseases observed in populations whose diets consist mainly of improperly processed cassava, which involves high cyanide exposure.

There is a need for more extensive occurrence data for cyanogenic glycosides. These include data showing the ratio of cyanogenic glycosides to cyanohydrins to HCN in raw and processed versions of a range of foods containing cyanogenic glycosides. More occurrence data for foods other than cassava are needed, as are occurrence data for all foods from a broader range of countries around the world. Concentrations in foods as ready to consume would enable more accurate estimates

of dietary exposure to be undertaken. Individual data points from analytical surveys would be of use to evaluate distributions of cyanogenic glycosides in foods and to define adequate sampling protocols. Distributions of occurrence data could then be used for probabilistic dietary exposure assessments.

More consumption data for cassava and cassava products from a broader range of countries would enable more detailed estimates of dietary exposure to be conducted or refined. More acute and chronic dietary exposure assessments from a broader range of countries, particularly African countries, would enable a better estimation of the global risk of dietary exposure to cyanogenic glycosides.

13. REFERENCES

Abuye C, Berhane Y, Ersumo T (2008). The role of changing diet and altitude on goitre prevalence in five regional states in Ethiopia. *East African Journal of Public Health*, 5:163–168.

Adamolekun B (2010). Etiology of konzo, epidemic spastic paraparesis associated with cyanogenic glycosides in cassava: role of thiamine deficiency? *Journal of Neurological Sciences*, 296:30–33.

Adamolekun B (2011). Neurological disorders associated with cassava diet: a review of putative etiological mechanisms. *Metabolic Brain Disease*, 26:79–85.

Adewusi SRA, Oke OL (1985). On the metabolism of amygdalin. 1. The LD_{50} and biochemical changes in rats. *Canadian Journal of Physiology and Pharmacology*, 63:1080–1083.

Adindu MN, Olayemi FF, Nze-Dike OU (2003). Cyanogenic potential of some cassava products in Port Harcourt markets in Nigeria. *Journal of Food Composition and Analysis*, 16:21–24.

Agbor-Egbe T, Lape Mbome I (2006). The effects of processing techniques in reducing cyanogen levels during production of some Cameroonian cassava foods. *Journal of Food Composition and Analysis*, 19(4):354–363.

Akanji AO, Famuyiwa OO (1993). The effects of chronic cassava consumption, cyanide intoxication and protein malnutrition on glucose tolerance in growing rats. *British Journal of Nutrition*, 69:269–276.

Aletor VA (1993). Cyanide in garri. Distribution of total, bound and free hydrocyanic acid in commercial garri, and the effect of fermentation time on residual cyanide content. *International Journal of Food Sciences and Nutrition*, 44:281–287.

Alonso-Amelot ME, Oliveros A (2000). A method for practical quantification and kinetic evaluation of the cyanogenesis in plant material. Application to *Pteridium quilinum* and *Passiflora capsularis*. *Phytochemical Analysis*, 11:309–316.

Amarowicz R, Chong X, Shahidi F (1993). Chromatographic techniques for preparation of linustatin and neolinustatin from flaxseed: standards for glycoside analyses. *Food Chemistry*, 48:99–101.

Amarowicz R, Wanasundara PKJPD, Shahidi F (1993). TLC separation of linamarin, linustatin and neolinustatin. *Die Nahrung*, 37:88–90.

Aminlari M, Vaseghi T, Kargar MA (1994). The cyanide-metabolizing enzyme rhodanese in different parts of the respiratory systems in sheep and dog. *Toxicology and Applied Pharmacology*, 124:64–71.

Ansell M, Lewis FA (1970). A review of cyanide concentrations found in human organs. A survey of literature concerning cyanide metabolism, "normal", non-fatal, and fatal body cyanide levels. *Journal of Forensic Medicine*, 17(4):148–155.

ATSDR (2006). *Toxicological profile for cyanide.* Atlanta, GA, USA, Department of Health and Human Services, Public Health Service, Agency for Toxic Substances and Disease Registry (http://www.atsdr.cdc.gov/ToxProfiles/tp8.pdf).

Bacala R, Barthet V (2007). Development of extraction and gas chromatography analytical methodology for cyanogenic glycosides in flaxseed (*Linum usitatissimum*). *Journal of AOAC International*, 90:153–161.

Bak S et al. (2006). Cyanogenic glycosides: a case study for evolution and application of cytochromes P450. *Phytochemistry Reviews*, 5:309–329.

Ballantyne B (1988). Toxicology and hazard evaluation of cyanide fumigation powders. *Journal of Toxicology. Clinical Toxicology*, 26(5–6):325–335.

Banea-Mayambu JP et al. (1997). Geographical and seasonal association between linamarin and cyanide exposure from cassava and the upper motor neurone disease konzo in former Zaire. *Tropical Medicine & International Health*, 2:1143–1151.

Banea-Mayambu J-P et al. (2000). Dietary cyanide from insufficiently processed cassava and growth retardation in children in the Democratic Republic of Congo (formerly Zaire). *Annals of Tropical Paediatrics*, 20:34–40.

Barceloux DG (2009). Cyanogenic foods (cassava, fruit kernels, and cycad seeds). *Disease-a-Month*, 55:336–352.

Barlow S et al. (2006). Risk assessment of substances that are both genotoxic and carcinogenic—Report of an international conference organised by EFSA and WHO with support of ILSI Europe. *Food and Chemical Toxicology*, 44:1636–1650.

Barrett MD et al. (1977). Fate of orally dosed linamarin in the rat. *Canadian Journal of Physiology and Pharmacology,* 55:134–136.

Barthet V, Bacala R (2010). Development of optimized extraction methodology for cyanogenic glycosides from flaxseed (*Linum usitatissimum*). *Journal of AOAC International*, 93:478–484.

Benford D et al. (2010). Application of the margin of exposure (MOE) approach to substances in food that are genotoxic and carcinogenic. *Food and Chemical Toxicology*, 48:S2–S24.

Billaut-Laden I et al. (2006). Evidence for a functional genetic polymorphism of the human thiosulfate sulfurtransferase (rhodanese), a cyanide and H_2S detoxification enzyme. *Toxicology*, 225(1):1–11.

Bissett FH et al. (1969). Cyanogenesis in manioc: concerning lotaustralin. *Phytochemistry*, 8:2235–2247.

Boby RG, Indira M (2003). The impact of cyanoglycoside rich fraction isolated from cassava (*Manihot esculenta*) on alcohol induced oxidative stress. *Toxicon*, 42(4):367–372.

Boby RG, Indira M (2004). Effect of co-administration of cassava (*Manihot esculenta* Crantz) rich diet and alcohol in rats. *Indian Journal of Physiology and Pharmacology*, 48(1):41–50.

Boccas B (1987). Cassava: staple food crop of prime importance in the tropics. *Courier*, 101:72–73.

Bokanga M et al. (1994). Genotype–environment interactions for cyanogenic potential in cassava. *Acta Horticulturae*, 375:131–139.

Bonmarin I, Nunga M, Perea WA (2002). Konzo outbreak, in the south-west of the Democratic Republic of Congo, 1996. *Journal of Tropical Pediatrics*, 48:234–238.

Bradbury JH (2006). Simple wetting method to reduce cyanogen content of cassava flour. *Journal of Food Composition and Analysis*, 19:388–393.

Bradbury JH (2009). Development of a sensitive picrate method to determine total cyanide and acetone cyanohydrin contents of gari from cassava. *Food Chemistry*, 113:1329–1333.

Bradbury JH, Denton IC (2010a). Simple method to reduce the cyanogen content of gari made from cassava. *Food Chemistry*, 123:840–845.

Bradbury JH, Denton IC (2010b). Rapid wetting method to reduce cyanogen content of cassava flour. *Food Chemistry*, 121:591–594.

Bradbury JH, Bradbury MG, Egan SV (1994). Comparison of methods of analysis of cyanogens in cassava. *Acta Horticulturae*, 375:87–96.

Bradbury JH, Cliff J, Denton IC (2011). Uptake of wetting method in Africa to reduce cyanide poisoning and konzo from cassava. *Food Chemistry and Toxicology*, 49:539–542.

Bradbury JH, Egan SV, Lynch MJ (1991). Analysis of cyanide in cassava using acid hydrolysis of cyanogenic glucosides. *Journal of the Science of Food and Agriculture*, 55:277–290.

Bradbury JH, Egan SV, Lynch MJ (1992). Rapid screening assay of cyanide content of cassava. *Phytochemical Analysis*, 3:91–94.

Bradbury MG, Egan SV, Bradbury JH (1999). Picrate paper kits for determination of total cyanogens in cassava roots and all forms of cyanogens in cassava products. *Journal of the Science of Food and Agriculture*, 79(4):593–601.

Brimer L, Rosling H (1993). Microdiffusion method with solid state detection of cyanogenic glycosides from cassava in human urine. *Food and Chemical Toxicology*, 31(8):599–603.

Brimer L et al. (1983). Determination of cyanogenic compounds by thin layer chromatography. 1. A densitometric method of quantification of cyanogenic glycosides, employing enzyme preparations from *Helix pomatia* and picrate-impregnated ion exchange sheets. *Journal of Agricultural and Food Chemistry*, 31:789–793.

Brinker AM, Seigler DS (1992). Determination of cyanide and cyanogenic glycosides from plants. In: Linskens HF, Jackson JF, eds. *Plant toxin analysis*. Berlin, Germany, Springer-Verlag, pp. 359–381 (Modern Methods of Plant Analysis, New Series Volume 13).

Butler GW, Bailey RW, Kennedy LD (1965). Studies on the glucosidase linamarase. *Phytochemistry*, 4:369–381.

Cairns T et al. (1978). Analytical chemistry of amygdalin. *Analytical Chemistry*, 50:317–322.

Campa C et al. (2000). Analysis of cyanogenic glycosides by micellar capillary electrophoresis. *Journal of Chromatography*, 739:95–100.

Cardoso AP et al. (1998). Cyanogenic potential of cassava flour: field trial in Mozambique of a simple kit. *International Journal of Food Sciences and Nutrition*, 49:93–99.

Cardoso AP et al. (2004). Combination of cassava flour cyanide and urinary thiocyanate measurements of school children in Mozambique. *International Journal of Food Sciences and Nutrition*, 55(3):183–190.

Cardoso AP et al. (2005). Processing of cassava roots to remove cyanogens. *Journal of Food Composition and Analysis*, 18:451–460.

Carella F et al. (1988). Dystonic-Parkinsonian syndrome after cyanide poisoning: clinical and MRI findings. *Journal of Neurology, Neurosurgery, and Psychiatry*, 51(10):1345–1348.

Carlsson L et al. (1999). Metabolic fates in humans of linamarin in cassava flour ingested as stiff porridge. *Food and Chemical Toxicology*, 37:307–312.

Carter JH, McLafferty MA, Goldman P (1980). Role of the gastrointestinal microflora in amygdalin (laetrile)-induced cyanide toxicity. *Biochemical Pharmacology*, 29:301–304.

CEC (1988). Council [of the European Communities] Directive of 22 June 1988 on the approximation of the laws of the Member States relating to flavourings for use in foodstuffs and to source materials for their production (88/388/EEC). *Official Journal*, L 184:61 (http://ec.europa.eu/food/fs/sfp/addit_flavor/flav09_en.pdf).

Chadha RK, Lawrence JF, Ratnayake WMN (1995). Ion chromatographic determination of cyanide released from flaxseed under autohydrolysis conditions. *Food Additives and Contaminants*, 12:527–533.

Chandra AK et al. (2004). Effect of bamboo shoot, *Bambusa arundinacea* (Retz.) Willd. on thyroid status under conditions of varying iodine intake in rats. *Indian Journal of Experimental Biology*, 42(8):781–786.

Chapin RE et al. (1993). The effects of feed restriction on reproductive function in Sprague-Dawley rats. *Fundamental and Applied Toxicology*, 20:23–29.

Chassagne D et al. (1996). Identification and quantification of passion fruit cyanogenic glycosides. *Journal of Agricultural and Food Chemistry*, 44(12):3817–3820.

Chiwona-Karltun L et al. (2000). Low dietary cyanogen exposure from frequent consumption of potentially toxic cassava in Malawi. *International Journal of Food Sciences and Nutrition*, 51:33–43.

Chiwona-Karltun L et al. (2004). Bitter taste in cassava roots correlates with cyanogenic glucoside levels. *Journal of the Science of Food and Agriculture*, 84(6):581–590.

Cho A-Y et al. (2006). Detection of abnormally high amygdalin content in food by an enzyme immunoassay. *Molecules and Cells*, 21:308–313.

Cho AY et al. (2008). A sensitive enzyme immunoassay for amygdalin in food extracts using a recombinant antibody. *Journal of Food Protection*, 71:2048–2052.

Cliff J et al. (1985). Association of high cyanide and low sulphur intake in cassava-induced spastic paraparesis. *Lancet*, 2(8466):1211–1213.

Cliff J et al. (1997). Konzo associated with war in Mozambique. *Tropical Medicine & International Health*, 2(11):1068–1074.

Cliff J et al. (1999). Ankle clonus and thiocyanate, linamarin, and inorganic sulphate excretion in school children in communities with Konzo, Mozambique. *Journal of Tropical Pediatrics*, 45:139–142.

Cliff J et al. (2011). Konzo and continuing cyanide intoxication from cassava in Mozambique. *Food and Chemical Toxicology*, 49:631–635.

CoE (2005). Hydrocyanic acid [datasheet]. In: *Active principles (constituents of toxicological concern) contained in natural sources of flavourings*. Approved by the 54th meeting of the Committee of Experts on Flavouring Substances, Council of Europe, Strasbourg, France, October, pp. 97–107 (http://www.coe.int/t/e/social_cohesion/soc-sp/public_health/flavouring_substances/Active%20principles.pdf).

Committee on Toxicity (2006). *Statement on cyanogenic glycosides in bitter apricot kernels. COT Statement 2006/15*. London, United Kingdom, Food Standards Agency, Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment, December (http://cot.food.gov.uk/pdfs/cotstatementapricot200615.pdf).

Conn EE (1980). Cyanogenic compounds. *Annual Review of Plant Physiology*, 31:433–451.

Cooke RD (1978). An enzymic assay for the total cyanide content of cassava (*Manihot esculenta* Crantz). *Journal of the Science of Food and Agriculture*, 29:345–352.

Cunnane SC et al. (1993). High alpha-linolenic acid flaxseed (*Linum usitatissimum*): some nutritional properties in humans. *British Journal of Nutrition*, 69:443–453.

Curtis AJ, Grayless CC, Fall R (2002). Simultaneous determination of cyanide and carbonyls in cyanogenic plants by gas chromatography–electron capture/photoionization detection. *Analyst*, 127:1446–1449.

Damardjati DS, Widowati S, Rachim A (1993). Cassava flour production and consumers' acceptance at village level in Indonesia. *Indonesian Agricultural Research and Development Journal*, 15(1):16–25.

Delange F, Ekpechi LO, Rosling H (1994). Cassava cyanogenesis and iodine deficiency disorders. *Acta Horticulturae*, 375(Suppl.):289–294.

Diasolua Ngudi D et al. (2011). Konzo and dietary pattern in cassava-consuming populations of Popokabaka, Democratic Republic of Congo. *Food and Chemical Toxicology*, 49:613–619.

Dixon AGO, Asiedu R, Bokanga M (1994). Breeding of cassava for low cyanogenic potential: problems, progress and prospects. *Acta Horticulturae*, 375:153–161.

Djazuli M, Bradbury JH (1999). Cyanogen content of cassava roots and flour in Indonesia. *Food Chemistry*, 65:523–525.

Doherty PA, Ferm VH, Smith RP (1982). Congenital malformations induced by infusion of sodium cyanide in the Golden hamster. *Toxicology and Applied Pharmacology*, 64(3):456–464.

EFSA (2004). Opinion of the Scientific Panel on Food Additives, Flavourings, Processing Aids and Materials in Contact with Food (AFC) on hydrocyanic acid in flavourings and other food ingredients with flavouring properties. *EFSA [European Food Safety Authority] Journal*, 105:1–28.

EFSA (2007a). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the Commission related to cyanogenic compounds as undesirable substances in animal feed. *EFSA [European Food Safety Authority] Journal*, 434:1–67.

EFSA (2007b). Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the European Commission on ethyl carbamate and hydrocyanic acid in food and beverages. *EFSA [European Food Safety Authority] Journal*, 551:1–44.

Ellington JJ, Stancil FE, Payne WD (1987). *Measurement of hydrolysis rate constants for evaluation of hazardous waste land disposal. Vol. 1. Data on 32 chemicals.* Washington, DC, USA, Environmental Protection Agency, Environmental Research Laboratory (EPA 600/3-86/043; NTIS PB87-140349).

Ernesto M et al. (2000). Cyanogens in cassava flour and roots and urinary thiocyanate concentration in Mozambique. *Journal of Food Composition and Analysis*, 13:1–12.

Ernesto M et al. (2002a). Persistent konzo and cyanogen toxicity from cassava in northern Mozambique. *Acta Tropica*, 82:357–362.

Ernesto M et al. (2002b). Strategy for the elimination of konzo in Mozambique. *Roots*, 8:8–11 (http://www.anu.edu.au/BoZo/CCDN/papers/8_8_11_02_b.pdf).

ESR (2010). *Survey of cyanogenic glycosides in plant-based foods.* Christchurch, New Zealand, Institute of Environmental Science & Research Limited (unpublished report).

Essers AJA (1994). Further improving the enzymic assay for cyanogens in cassava products. *Acta Horticulturae*, 375:97–104.

Essers AJA et al. (1993). Studies on the quantification of specific cyanogens in cassava products and introduction of a new chromogen. *Journal of the Science of Food and Agriculture*, 63:287–296.

Eyjolfsson R (1970). Recent advances in the chemistry of cyanogenic glycosides. *Fortschritte der Chemie organischer Naturstoffe*, 27:74–108.

Ezeji EU et al. (2009). Effect of gari diet on marker enzymes of mice liver mitochondria. *Pakistan Journal of Nutrition*, 8(4):414–418.

Fan TW-M, Conn EE (1985). Isolation and characterization of two cyanogenic β-glucosidases from flax seeds. *Archives of Biochemistry and Biophysics*, 243:361–373.

FAO/WHO (1965). *Report of the second joint meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues.* Rome, Italy, Food and Agriculture Organization of the United Nations; Geneva, Switzerland, World Health Organization (FAO Meeting Report No. PL/1965/10; WHO/Food Add./26.65).

FAO/WHO (2002). Further guidance on derivation of the ARfD. In: *Pesticide residues in food—2002. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group on Pesticide Residues.* Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, 2002, pp. 4–8 (FAO Plant Production and Protection Paper 172).

FAO/WHO (2007). *Report of the Fifty-ninth Session of the Executive Committee of the Codex Alimentarius Commission, Rome, 26–29 June 2007.* Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (ALINORM 07/30/3; http://www.codexalimentarius.net/web/archives.jsp?year=07).

FAO/WHO (2008a). *Report of the Second Session of the Codex Committee on Contaminants in Foods, The Hague, the Netherlands, 31 March – 4 April 2008.* Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (ALINORM 08/32/41).

FAO/WHO (2008b). *Discussion paper on cyanogenic glycosides*. Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Codex Committee on Contaminants in Foods (CX/CF 09/3/11).

FAO/WHO (2009a). *Report of the Third Session of the Codex Committee on Contaminants in Foods, Rotterdam, the Netherlands, 23–27 March 2009.* Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission (ALINORM 09/32/41).

FAO/WHO (2009b). Dietary exposure assessment of chemicals in food. In: *Principles and methods for the risk assessment of chemicals in food.* Geneva, Switzerland, Food and Agriculture Organization of the United Nations and World Health Organization (Environmental Health Criteria 240).

Feigl F, Anger V (1966). Replacement of benzidine by copper ethylacetoacetate and tetra base as spot-test reagent for hydrogen cyanide and cyanogen. *Analyst*, 91:282–284.

FERG (2010). *Incidence and prevalence estimates of cassava-cyanide-induced diseases. Draft report for the fourth formal meeting of the Foodborne Disease Burden Epidemiology Reference Group (FERG).* Geneva, Switzerland, World Health Organization.

Ferreira VLP, Yotsuyanagi K, Carvalho CRL (1995). Elimination of cyanogenic compounds from bamboo shoots *Dendrocalamus giganteus* Munro. *Tropical Science*, 35:342–346.

Fokunang CN et al. (2001). Cyanogenic potential in food crops and its implication in cassava (*Manihot esculenta* Crantz) production. *Pakistan Journal of Biochemical Sciences*, 4(7):926–930.

Frakes RA, Sharma RP, Willhite CC (1985). Developmental toxicity of the cyanogenic glycoside linamarin in the Golden hamster. *Teratology*, 31:241–246.

Frakes RA, Sharma RP, Willhite CC (1986). Comparative metabolism of linamarin and amygdalin in hamsters. *Food and Chemical Toxicology*, 24(5):417–420.

Frakes RA et al. (1986). Effect of cyanogenic glycosides and protein content in cassava diets on hamster prenatal development. *Fundamental and Applied Toxicology*, 7:191–198.

Frehner M, Scalet M, Conn EE (1990). Pattern of the cyanide-potential in developing fruits: implications for plants accumulating cyanogenic monoglucosides (*Phaseolus lunatus*) or cyanogenic diglucosides in their seeds (*Linum usitatissimum, Prunus amygdalus*). *Plant Physiology*, 94:28–34.

FSANZ (2004a). *Final assessment report. Proposal P257. Advice on the preparation of cassava and bamboo shoots.* Canberra, Australia, Food Standards Australia New Zealand, 17 March.

FSANZ (2004b). *Cyanogenic glycosides in cassava and bamboo shoots. A human health risk assessment.* Canberra, Australia, Food Standards Australia New Zealand, July (Technical Report Series No. 28).

FSANZ (2008a). *Proposal P1002—Hydrocyanic acid in ready-to-eat cassava chips. Assessment report.* Canberra, Australia, Food Standards Australia New Zealand, 6 March (http://www.foodstandards.gov.au/_srcfiles/P1002_Cassava_in_Vege_chips_AR.pdf).

FSANZ (2008b). *Proposal P1002—Hydrocyanic acid in ready-to-eat cassava chips. Approval report.* Canberra, Australia, Food Standards Australia New Zealand, 15 September (http://www.foodstandards.gov.au/_srcfiles/P1002%20Hydrocyanic%20acid%20in%20cassava%20chips%20AppR%20FINAL.pdf#search=%22p1002%22).

FSANZ (2009). *Hydrocyanic acid in ready-to-eat cassava chips. First review report.* Canberra, Australia, Food Standards Australia New Zealand (Report No. 2-09).

FSANZ (2011). *Australia New Zealand Food Standards Code. Up to and including Amendment 121.* Canberra, Australia, Food Standards Australia New Zealand.

Ganjewala D et al. (2010). Advances in cyanogenic glycosides biosynthesis and analyses in plants: a review. *Acta Biologica Szegediensis*, 54(1):1–14.

Geller RJ et al. (2006). Pediatric cyanide poisoning: causes, manifestations, management, and unmet needs. *Pediatrics*, 118:2146–2158.

Gettler AO, Baine JO (1938). The toxicology of cyanide. *American Journal of the Medical Sciences*, 195:182–198.

Gupta YP (1987). Anti-nutritional and toxic factors in food legumes: a review. *Plant Foods for Human Nutrition*, 37(3):201–228.

Haorongbam S, Elangbam D, Nirmala C (2009). Cyanogenic glucosides in juvenile edible shoots of some Indian bamboos. In: *Proceedings of the VIII World Bamboo Conference, Bangkok, Thailand. Vol. 6.* pp. 22–30 (http://www.bambusc.org.br/wp-content/gallery/WBC2009/WBCVIII-Vol_06.pdf).

Haque RM, Bradbury HJ (2002). Total cyanide determination of plants and foods using the picrate and acid hydrolysis methods. *Food Chemistry*, 77(1):107–114.

Hernandez T et al. (1995). Fate in humans of dietary intake of cyanogenic glycosides from roots of sweet cassava consumed in Cuba. *Natural Toxins*, 3(2):114–117.

Himwich WA, Saunders JP (1948). Enzymatic conversion of cyanide to thiocyanate. *American Journal of Physiology*, 153(2):348–354.

ICI (1993). *Kinetics for the dissociation of acetone cyanohydrin in water*. London, United Kingdom, Imperial Chemical Co.

Ihedioha JI, Chineme CN (2003). Clinicopathologic implications of shortened fermentation periods in the production of toasted cassava granules (gari). *Plant Foods for Human Nutrition*, 58:1–11.

Imosemi IO et al. (2005). Gross morphological studies on the effect of cyanide on the developing cerebellum of Wistar rat (*Rattus norvegicus*). *African Journal of Medicine and Medical Sciences*, 34(1):59–63.

IPCS (2004). *Hydrogen cyanide and cyanides: human health aspects*. Geneva, Switzerland, World Health Organization, International Programme on Chemical Safety (Concise International Chemical Assessment Document 61).

Jackson LC (1988). Behavioral effects of chronic sublethal dietary cyanide in an animal model: implications for humans consuming cassava (*Manihot esculenta*). *Journal of the Society for the Study of Human Biology*, 60:597–614.

Jones DA (1997). Why are so many food plants cyanogenic? *Phytochemistry*, 47:155–162.

Jørgensen K et al. (2011). Biosynthesis of the cyanogenic glucosides linamarin and lotaustralin in cassava: isolation, biochemical characterization, and expression pattern of CYP71E7, the oxime-metabolizing cytochrome P450 enzyme. *Plant Physiology*, 155:282–292.

Kadushin FS et al. (1988). Cyanide induced parkinsonism: neuropsychological and radiological findings. *Veterinary and Human Toxicology*, 30:359 (abstract).

Kamalu BP (1993). Pathological changes in growing dogs fed on a balanced cassava (*Manihot esculenta* Crantz) diet. *British Journal of Nutrition*, 69:921–934.

Kamalu BP, Agharanya JC (1991). The effect of a nutritionally-balanced cassava (*Manihot esculenta* Crantz) diet on endocrine function using the dog as a model. 2. Thyroid. *British Journal of Nutrition*, 65(3):373–379.

Kassa RM et al. (2011). On the biomarkers and mechanisms of konzo, a distinct upper motor neuron disease associated with food (cassava) cyanogenic exposure. *Food and Chemical Toxicology*, 49:571–578.

Kendirim OC, Chukwu OA, Achinewhu SC (1995). Effect of traditional processing of cassava on the cyanide content of gari and cassava flour. *Plant Foods for Human Nutrition*, 48:335–339.

Keusgen M et al. (2004). Direct determination of cyanides by potentiometric biosensors. *Sensors & Actuators, B*, 103:380–385.

Kim Y-S et al. (2008). Biotransformation of ginsenoside Rb1, crocin, amygdalin, geniposide, puerarin, ginsenoside Re, hesperidin, poncirin, glycyrrhizin, and baicalin by human fecal microflora and its relation to cytotoxicity against tumor cells. *Journal of Microbiology and Biotechnology*, 18(6):1109–1114.

Kittivachra R (2006). Effects of cassava on thyroid gland in rats. *Thai Journal of Pharmaceutical Sciences*, 30:57–62.

Kobaisy M, Oomah BD, Mazza G (1996). Determination of cyanogenic glycosides in flaxseed by barbituric acid–pyridine, pyridine–pyrazolone, and high-performance liquid chromatography methods. *Journal of Agricultural and Food Chemistry*, 44(10):3178–3181.

Kobawila SC et al. (2005). Reduction of the cyanide content during fermentation of cassava roots and leaves to produce bikedi and ntoba mbodi, two food products from Congo. *African Journal of Biotechnology*, 4(7):689–696.

Kolodziejczyk PP, Fedec P (1995). Processing flaxseed for human consumption. In: Cunnane SC, Thompson LU, eds. *Flaxseed and human nutrition*, 1st ed. Champaign, IL, AOCS Press, pp. 261–280.

König W (1904). Über eine neue, vom Pyridin derivierende Klasse von Farbstoffen. *Journal für Praktische Chemie*, 69:105–137 (in German).

Krech MJ, Fieldes MA (2003). Analysis of the developmental regulation of the cyanogenic compounds in seedlings of two lines of *Linum usitatissimum* L. *Canadian Journal of Botany*, 81:1029–1038.

Kupchella L, Syty A (1984). Determination of cyanogenic glycoside in seeds by molecular absorption spectrometry in the gas phase. *Journal of the Association of Official Analytical Chemists*, 67(1):188–191.

Kuti JO, Konoru HB (2006). Cyanogenic glycosides content in two edible leaves of tree spinach (*Cnidoscolus* spp.). *Journal of Food Composition and Analysis*, 19:556–561.

Lagasse R et al. (1980). Endemic goitre and cretinism in Ubangi. In: Ermans AM et al., eds. *Role of cassava in the etiology of endemic goitre and cretinism*. Ottawa, Ontario, Canada, International Development Research Centre, pp. 45–60 (http://idl-bnc.idrc.ca/dspace/bitstream/10625/4287/1/38090.pdf).

Lambert JL, Ramasamy J, Paukstelis JV (1975). Stable reagents for the colorimetric determination of cyanide by modified König reactions. *Analytical Chemistry*, 47:916–918.

Laurena AC, Revilleza MJR, Mendoza EMT (1994). Polyphenols, phytate, cyanogenic glycosides, and trypsin inhibitor activity of several Philippine indigenous food legumes. *Journal of Food Composition and Analysis*, 7(3):194–202.

Lautie JP, Stankovic V, Sinoquet G (2000). Determination of chlormequat in pears by high-performance thin layer chromatography and high-performance liquid chromatography with conductimetric detection. *Analysis*, 28:155–158.

Leuschner J, Winkler A, Leuschner F (1991). Toxicokinetic aspects of chronic cyanide exposure in the rat. *Toxicology Letters*, 57(2):195–201.

Leuschner F et al. (1989). *13-week toxicity study of potassium cyanide administered to Sprague-Dawley rats in the drinking water.* Hamburg, Federal Republic of Germany, Laboratory of Pharmacology and Toxicology, July (unpublished study).

Linden CH, Lovejoy FH Jr (1998). Poisoning and drug overdose. In: Fauci AS, Braunwald E, Isselbacher KJ, eds. *Harrison's principles of internal medicine*. New York, NY, USA, McGraw-Hill Health Professions Division [cited in ATSDR, 2006].

Llorens J et al. (2011). A new unifying hypothesis for lathyrism, konzo and tropical ataxic neuropathy: nitriles are the causative agents. *Food and Chemical Toxicology*, 49:563–570.

Lundquist P, Rosling H, Sörbo B (1985). Determination of cyanide in whole blood, erythrocytes, and plasma. *Clinical Chemistry*, 31:591–595.

Lundquist P et al. (1979). Method for determining thiocyanate in serum and urine. *Clinical Chemistry*, 2:678–681.

Madhusudanan M et al. (2008). Clinical and etiological profile of tropical ataxic neuropathy in Kerala, South India. *European Neurology*, 60:21–26.

Maduagwu EN (1989). Metabolism of linamarin in rats. *Food and Chemical Toxicology*, 27(7):451–454.

Mak KKW et al. (2005). Application of cyanide hydrolase from *Klebsiella* sp. in a biosensor system for the detection of low-level cyanide. *Applied Microbiology and Biotechnology*, 67:631–636.

Malomo AO et al. (2004). Histomorphometric studies on the effect of cyanide consumption of the developing cerebellum of Wistar rat (*Rattus norvegicus*). *West African Journal of Medicine*, 23(4):323–328.

Manzano H et al. (2007). Effects of long-term cyanide ingestion by pigs. *Veterinary Research Communications*, 31(1):93–104.

Mathangi DC, Namasivayam A (2000a). Effect of chronic cyanide intoxication on memory in albino rats. *Food and Chemical Toxicology*, 38:51–55.

Mathangi DC, Namasivayam A (2000b). Effect of cassava consumption on open-field behavior and brain neurotransmitters in albino rats. *Physiology & Behavior*, 70:89–93.

Mathangi DC, Mohan V, Namasivayam A (1999). Effect of cassava on motor co-ordination and neurotransmitter level in the albino rat. *Food and Chemical Toxicology*, 37:57–60.

Mathangi DC et al. (2000). Long-term ingestion of cassava (tapioca) does not produce diabetes or pancreatitis in the rat model. *International Journal of Pancreatology*, 27(3):203–208.

Mathangi DC et al. (2011). Effect of alpha-ketoglutarate on neurobehavioral, neurochemical and oxidative changes caused by sub-chronic cyanide poisoning in rats. *Neurochemical Research*, 36:540–548.

McMahon JM, White WLB, Sayre RT (1995). Cyanogenesis in cassava (*Manihot esculenta* Crantz). *Journal of Experimental Botany*, 46:731–741.

McMillan DE, Geevarghese PJ (1979). Dietary cyanide in tropical malnutrition diabetes. *Diabetes Care*, 2(2):202–208.

Mendoza EMT et al. (1984). Evaluation of some methods for the analysis of cyanide in cassava. In: Uritani I, Reyes ED, eds. *Tropical root crops: postharvest physiology and processing*. Tokyo, Japan, Japan Scientific Societies Press, pp. 235–242.

Ministry of Health Mozambique (1984). Mantakassa: an epidemic of spastic paraparesis associated with chronic cyanide intoxication in a cassava staple area of Mozambique. 1. Epidemiology and clinical and laboratory findings in patients. *Bulletin of the World Health Organization*, 62(3):447–484.

Mkumbira J et al. (2003). Classification of cassava into "bitter" and "cool" in Malawi: from farmers' perception to characterisation by molecular markers. *Euphytica*, 132(1):7–22.

Mlingi NLV, Bainbridge Z (1994). Reduction of cyanogen levels during sun-drying of cassava in Tanzania. *Acta Horticulturae*, 375:233–239.

Mlingi N, Poulter NH, Rosling H (1992). An outbreak of acute intoxications from consumption of insufficiently processed cassava in Tanzania. *Nutrition Research*, 12:677–687.

Mlingi NLV et al. (1995). Critical stages in cyanogen removal during cassava processing in southern Tanzania. *Food Chemistry*, 53:29–33.

Mlingi NLV et al. (1996). Milling reduces the goitrogenic potential of cassava. *International Journal of Food Sciences and Nutrition*, 47:445–454.

Mlingi N et al. (1998). Low cyanide exposure from consumption of cassava in Dar es Salaam, Tanzania. *Natural Toxins*, 6:67–72.

Mlingi NLV et al. (2011). Recurrence of konzo in southern Tanzania: rehabilitation and prevention using the wetting method. *Food and Chemical Toxicology*, 49:673–677.

Moertel CG et al. (1981). A pharmacologic and toxicological study of amygdalin. *JAMA*, 245:591–594.

Moertel CG et al. (1982). A clinical trial of amygdalin (laetrile) in the treatment of human cancer. *New England Journal of Medicine*, 306:201–206.

Montagnac JA, Davis CR, Tanumihardjo SA (2009). Processing techniques to reduce toxicity and antinutrients of cassava for use as a staple food. *Comprehensive Reviews in Food Science and Food Safety*, 8:17–27.

Morant AV, Jørgensen K (2008). β-Glucosidases as detonators of plant chemical defense. *Phytochemistry*, 69:1795–1813.

Murphy KE et al. (2006). Determination of cyanide in blood by isotope-dilution gas chromatography–mass spectrometry. *Clinical Chemistry*, 52:4558–4567.

Nagashima S (1978). Spectrophotometric determination of cyanide with sodium isonicotinate and sodium barbiturate. *Analytica Chimica Acta*, 99:197–201.

Nahrstedt A (1970). Zur cyanogenese in *Prunus avium*. *Phytochemistry*, 9:2085–2089 (in German).

Nahrstedt A (1973). [Gas-chromatography of diastereomeric cyanogenic glycosides.] *Planta Medica*, 24:83–89 (in German).

Nambisan B (2011). Strategies for elimination of cyanogens from cassava for reducing toxicity and improving food safety. *Food and Chemical Toxicology*, 49:690–693.

Nambisan B, Sundaresan S (1985). Effect of processing on the cyanoglucoside content of cassava. *Journal of the Science of Food and Agriculture*, 36:1197–1203.

NAS (2009). Acetone cyanohydrin. In: *Acute exposure guidelines for selected airborne chemicals. Vol. 7.* Washington, DC, USA, National Academy of Sciences, Board on

Environmental Studies and Toxicology, pp. 13–49 (http://www.nap.edu/catalog/12503.html).

National Council on Nutrition and Physical Activity (1999). [*NORKOST 1997. National dietary survey among men and women 16–79 years of age.*] Oslo, Norway, National Council on Nutrition and Physical Activity (Report 3/1999) (in Norwegian).

Németh K et al. (2003). Deglycosylation by small intestinal epithelial cell beta-glucosidases is a critical step in the absorption and metabolism of dietary flavonoid glycosides in humans. *European Journal of Nutrition*, 42(1):29–42.

Newton GW et al. (1981). Amygdalin toxicity studies in rats predict chronic cyanide poisoning in humans. *Western Journal of Medicine*, 134:97–103.

Ngudi DD, Kuo Y-H, Lambein F (2002). Food safety and amino acid balance in processed cassava "cossettes". *Journal of Agricultural and Food Chemistry*, 50:3042–3049.

Ngudi DD, Kuo Y-H, Lambein F (2003). Cassava cyanogens and free amino acids in raw and cooked leaves. *Food and Chemical Toxicology*, 41:1193–1197.

Niedźwiedź-Siegieň I (1998). Cyanogenic glucosides in *Linum usitatissimum*. *Phytochemistry*, 49:59–63.

NTP (1993). *NTP technical report on toxicity studies of sodium cyanide (CAS No. 143-33-9) administered in drinking water to F344/N rats and B6C3F1 mice.* Research Triangle Park, NC, USA, Department of Health and Human Services, Public Health Service, National Toxicology Program (NTP Toxicity Report Series No. 37; NIH Publication 94-3386; http://ntp.niehs.nih.gov/ntp/htdocs/ST_rpts/tox037.pdf).

Nunn PB, Lyddiard JR, Perera KPWC (2011). Brain glutathione as a target for aetiological factors in neurolathyrism and konzo. *Food and Chemical Toxicology*, 49:662–667.

Nyirenda DB et al. (2011). Chemical safety of cassava products in regions adopting cassava production and processing—Experience from southern Africa. *Food and Chemical Toxicology*, 49(3):607–612.

NZFSA (2008). *Cyanogenic glycosides—information sheet.* Wellington, New Zealand, New Zealand Food Safety Authority (http://www.foodsafety.govt.nz/elibrary/industry/Cyanogenic_Glycosides-Toxin_Which.pdf; accessed 22 March 2011).

Obilie EM, Tano-Debrah K, Amoa-Awua WK (2004). Souring and breakdown of cyanogenic glycosides during the processing of cassava into akyeke. *International Journal of Food Microbiology*, 93(1):115–121.

O'Brien GM, Taylor AJ, Poulter NH (1991). Improved enzymic assay for cyanogens in fresh and processed cassava. *Journal of the Science of Food and Agriculture*, 56:277–289.

Okafor PN, Okorowkwo CO, Maduagwu EN (2002). Occupational and dietary exposures of humans to cyanide poisoning from large-scale cassava processing and ingestion of cassava foods. *Food and Chemical Toxicology*, 40:1001–1005.

Oke OL (1994). Eliminating cyanogens from cassava through processing: technology and tradition. *Acta Horticulturae*, 375:163–174.

Okolie NP, Osagie AU (1999). Liver and kidney lesions and associated enzyme changes induced in rabbits by chronic cyanide exposure. *Food and Chemical Toxicology*, 37(7):745–750.

Okolie NP, Osagie AU (2000). Differential effects of chronic cyanide intoxication on heart, lung and pancreatic tissues. *Food and Chemical Toxicology*, 38(6):543–548.

Ologhobo AD, Fetuga BL, Tewe OO (1984). The cyanogenic glycoside contents of raw and processed lima bean varieties. *Food Chemistry*, 13(2):117–128.

Oluwole OSA (2008). Cyanogenicity of cassava varieties and risk of exposure to cyanide from cassava food in Nigerian communities. *Journal of the Science of Food and Agriculture*, 88(6):962–969.

Oluwole OSA, Onabolu AO, Sowunmi A (2002). Exposure to cyanide following a meal of cassava food. *Toxicology Letters*, 135:19–23.

Oluwole OS et al. (2002). Low prevalence of ataxic polyneuropathy in a community with high exposure to cyanide from cassava foods. *Journal of Neurology*, 249:1034–1040.

Oluwole OS et al. (2003). Incidence of endemic ataxic polyneuropathy and its relation to exposure to cyanide in a Nigerian community. *Journal of Neurology, Neurosurgery, and Psychiatry*, 74:1417–1422.

Oluwole OSA et al. (2007). Characterisation of cassava (*Manihot esculenta* Crantz) varieties in Nigeria and Tanzania, and farmers' perception of toxicity of cassava. *Journal of Food Composition and Analysis*, 20(7):559–567.

Onabolu AO, Oluwole OSA, Bokanga M (2002). Loss of residual cyanogens in a cassava food during short-term storage. *International Journal of Food Sciences and Nutrition*, 53:343–349.

Onabolu A et al. (2001a). High cassava production and low dietary cyanide exposure in mid-west Nigeria. *Public Health Nutrition*, 4(1):3–9.

Onabolu AO et al. (2001b). Ecological variation of intake of cassava food and dietary cyanide load in Nigerian communities. *Public Health Nutrition*, 4(4):871–876.

Onabolu AO et al. (2002). Processing factors affecting the level of residual cyanohydrins in gari. *Journal of the Science of Food and Agriculture*, 82:966–969.

Oomah BD, Mazza G, Naschuk EO (1992). Cyanogenic compounds in flaxseed. *Journal of Agricultural and Food Chemistry*, 40(8):1346–1348.

Oyewole OI, Olayinka ET (2009). Hydroxocobalamin (vit b12a) effectively reduced extent of cyanide poisoning arising from oral amygdalin ingestion in rats. *Journal of Toxicology and Environmental Health Sciences*, 1(1):8–11.

Padmaja G (1995). Cyanide detoxification in cassava for food and feed use. *Critical Reviews in Food Science and Nutrition*, 35:229–239.

Palmer IS et al. (1980). Isolation of factors in linseed oil meal protective against chronic selenosis in rats. *Journal of Nutrition*, 110:145–150.

Park ER et al. (2005). Analysis and decrease of cyanogenic glycosides in flaxseed. *Journal of the Korean Society of Food Science and Nutrition*, 34:875–879.

Peterson S et al. (1995). Improved cassava-processing can help reduce iodine deficiency disorders in the Central African Republic. *Nutrition Research*, 15(6):803–812.

Philbrick DJ, Hill DC, Alexander JC (1977). Physiological and biochemical changes associated with linamarin administration to rats. *Toxicology and Applied Pharmacology*, 42:539–551.

Philbrick DJ, Hill DC, Alexander JC (1981). Influence of dose level and methionine intake on the effects of linamarin administration to rats. *Journal of Toxicology and Environmental Health*, 8(1–2):159–168.

Philbrick DJ et al. (1979). Effects of prolonged cyanide and thiocyanate feeding in rats. *Journal of Toxicology and Environmental Health*, 5(4):579–592.

RTECS (2004). *Hydrocyanic acid RTECS #: MW6825000*. Atlanta, GA, USA, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Education and Information Division, Registry of Toxic Effects of Chemical Substances.

Sadasivam S, Thayumanavan B (2003). Cyanogenic glycosides. In: *Molecular host plant resistance to pests*. New York, NY, USA, Marcel Dekker, pp. 132–163.

Sakata M et al. (1987). Toxicity of D,L-mandelonitrile-beta-D-glucoside, "prulaurasin" in rat. *Journal of Toxicological Sciences*, 12(1):47–55.

Sánchez-Pérez R et al. (2009). Tissue and cellular localization of individual β-glycosidases using a substrate-specific sugar reducing assay. *Plant Journal*, 60(5):894–906.

Sano A, Takimoto N, Takitani S (1992). High-performance liquid chromatographic determination of cyanide in human red blood cells by pre-column fluorescence derivatisation. *Journal of Chromatography*, 582:131–135.

Satya S et al. (2009). Exploring the nutraceutical potential and food safety aspects of bamboo shoot of some Indian species. In: *Proceedings of the VIII World Bamboo Conference, Bangkok, Thailand. Vol. 6*. pp. 78–88 (http://www.bambusc.org.br/wp-content/gallery/WBC2009/WBCVIII-Vol_06.pdf).

Schilcher VH, Wilkens-Sauer M (1986). *Quantitative Bestimmung Cyanogener Glykoside in* Linum usitatissimum *mit Hilfe der HPLC*. Fette, Seifen, Anstrichmittel, 8, pp. 287–290 (in German).

Schulz V, Bonn R, Kindler J (1979). Kinetics of elimination of thiocyanate in 7 healthy subjects and 8 subjects with renal failure. *Klinische Wochenschrifte*, 57:243–247.

Schulz V et al. (1982). Cyanide toxicity of sodium nitroprusside in therapeutic use with and without sodium thiosulfate. *Klinische Wochenschrift*, 60:1393–1400.

Seigler DS (1975). Isolation and characterization of naturally occurring cyanogenic compounds. *Phytochemistry*, 14:9–29.

Seigler DS (1991). Cyanide and cyanogenic glycosides. In: Rosenthal GA, Berenbaum MR, eds. *Herbivores: their interactions with secondary plant metabolites*, 2nd ed. *Vol. 1. The chemical participants.* San Diego, CA, USA, Academic Press, pp. 35–77.

Selmar D, Grocholewski S, Seigler DS (1990). Cyanogenic lipids: utilization during seedling development of *Ungnadia speciosa*. *Plant Physiology*, 93(2):631–636.

Selmar D, Lieberei R, Biehl B (1988). Mobilization and utilization of cyanogenic glycosides. *Plant Physiology*, 86:711–716.

Shahidi TG, Wanasundara PKJPD (1997). Cyanogenic glycosides of flaxseeds. *ACS Symposium Series*, 662:171–185.

Shibata M et al. (2004). Simultaneous determination of hydrogen cyanide and volatile aliphatic nitriles by headspace gas chromatography, and its application to an in vivo study of the metabolism of acrylonitrile in the rat. *Archives of Toxicology*, 78:301–305.

Shragg TA, Albertson TE, Fisher CJ (1982). Cyanide poisoning after bitter almond ingestion. *Western Journal of Medicine*, 136(1):65–69.

Simons-Gérard E et al. (1980). Foods consumed and endemic goitre in the Ubangi. In: Ermans AM et al., eds. *Role of cassava in the etiology of endemic goitre and cretinism*. Ottawa, Ontario, Canada, International Development Research Centre, pp. 69–80 (http://idl-bnc.idrc.ca/dspace/bitstream/10625/4287/1/38090.pdf).

Siritunga D, Sayre R (2007). Transgenic approaches for cyanogen reduction in cassava. *Journal of AOAC International*, 90(5):1450–1455.

Smith CR Jr et al. (1980). Linustatun and neolinustatin: cyanogenic glycosides of linseed meal that protect animals against selenium toxicity. *Journal of Organic Chemistry*, 45:507–510.

Sokari JG, Karibo PS (1992). Changes in cassava toxicity during processing into gari and ijapu—two fermented food products. *Food Additives and Contaminants*, 9(4):379–384.

Soler-Martín C et al. (2010). The targets of acetone cyanohydrin neurotoxicity in the rat are not the ones expected in an animal model of konzo. *Neurotoxicology and Teratology*, 32:289–294.

Soto-Blanco B, Górniak SL (2003). Milk transfer of cyanide and thiocyanate: cyanide exposure by lactation in goats. *Veterinary Research*, 34(2):213–220.

Soto-Blanco B, Górniak SL (2004). Prenatal toxicity of cyanide in goats—a model for teratological studies in ruminants. *Theriogenology*, 62(6):1012–1026.

Soto-Blanco B, Maiorka PC, Górniak SL (2002a). Effects of long-term low-dose cyanide administration to rats. *Ecotoxicology and Environmental Safety*, 53(1):37–41.

Soto-Blanco B, Maiorka PC, Górniak SL (2002b). Neuropathologic study of long term cyanide administration to goats. *Food and Chemical Toxicology*, 40(11):1693–1698.

Soto-Blanco B et al. (2008). Comparative effects of prolonged administration of cyanide, thiocyanate and chokecherry (*Prunus virginiana*) to goats. *Journal of Applied Toxicology*, 28:356–363.

Sousa AB et al. (2003). Toxicokinetics of cyanide in rats, pigs and goats after oral dosing with potassium cyanide. *Archives of Toxicology*, 77(6):330–334.

Spencer PS (1999). Food toxins, AMPA receptors, and motor neuron diseases. *Drug Metabolism Reviews*, 31:561–587.

Sreeja VG et al. (2003). New aspects in pathogenesis of konzo: neural cell damage directly caused by linamarin contained in cassava (*Manihot esculenta* Crantz). *British Journal of Nutrition*, 90:467–472.

Stochmal A, Oleszek W (1994). Determination of cyanogenic glucosides in white clover (*Trifolium repens* L.) by high performance liquid chromatography. *Phytochemical Analysis*, 5:271–272.

Strugala GJ, Rauws AG, Elbers R (1986). Intestinal first pass metabolism of amygdalin in the rat in vitro. *Biochemical Pharmacology*, 35(13):2123–2128.

Suchard JR, Wallace KL, Gerkin RD (1998). Acute cyanide toxicity caused by apricot kernel ingestion. *Annals of Emergency Medicine*, 32:742–744.

Sumiyoshi K, Yagi T, Nakamura H (1995). Determination of cyanide by high-performance liquid chromatography using postcolumn derivatization with *o*-phthalaldehyde. *Journal of Chromatography A*, 690:77–82.

Swain E, Li CP, Poulton JE (1992). Development of potential cyanogenesis in maturing black cherry (*Prunus serotina* Ehrh) fruits. *Plant Physiology*, 98:1423–1428.

Tewe OO, Maner JH (1980). Cyanide, protein and iodine interactions in the performance, metabolism and pathology of pigs. *Research in Veterinary Science*, 29:271–276.

Tewe OO, Maner JH (1981). Long-term and carry-over effect of dietary inorganic cyanide (KCN) in the life cycle performance and metabolism of rats. *Toxicology and Applied Pharmacology*, 58(1):1–7.

Tulsawani RK et al. (2005). Effect of sub-acute oral cyanide administration in rats: protective efficacy of alpha-ketoglutarate and sodium thiosulfate. *Chemico-Biological Interactions*, 156(1):1–12.

Tuncel G et al. (1990). Toxicological, nutritional and microbiological evaluation of tempe fermentation with *Rhizopu oligosporus* of bitter and sweet apricot seeds. *International Journal of Food Microbiology*, 11:337–344.

Tylleskär T et al. (1992). Cassava cyanogens and konzo, an upper motoneuron disease found in Africa. *Lancet*, 339(8787):208–211 [plus erratum in 339(8790):440].

Tylleskär T et al. (1994). Konzo in the Central African Republic. *Neurology*, 44:959–961.

Tylleskär T et al. (1995). Dietary determinants of a non-progressive spastic paraparesis (konzo): a case–referent study in a high incidence area of Zaire. *International Journal of Epidemiology*, 24(5):949–956.

Uitti RJ et al. (1985). Cyanide-induced parkinsonism: a clinicopathologic report. *Neurology*, 35(6):921–925.

USEPA (1990). *Summary review of health effects associated with hydrogen cyanide: health issue assessment*. Research Triangle Park, NC, USA, Environmental Protection Agency, Environmental Criteria and Assessment Office (EPA600890002F).

USEPA (2010). *Toxicological review of hydrogen cyanide and cyanide salts (CAS No. various) in support of summary information on the Integrated Risk Information System (IRIS)*. Washington, DC, USA, Environmental Protection Agency (http://www.epa.gov/iris/toxreviews/0060tr.pdf).

van Heijst AN et al. (1994). Chronic cyanide poisoning in relation to blindness and tropical neuropathy. *Journal of Toxicology. Clinical Toxicology*, 32:549–556.

Vetter J (2000). Plant cyanogenic glycosides. *Toxicon*, 38:11–36.

Wagner B, Galey WR (2003). Kinetic analysis of hexose transport to determine the mechanism of amygdalin and prunasin absorption in the intestine. *Applied Toxicology*, 23:371–375.

Wanasundara PKJPD, Shahidi F, Brosnan ME (1999). Changes in flax (*Linum usitatissimum*) seed nitrogenous compounds during germination. *Food Chemistry*, 65(3):289–295.

Wanasundara PKJPD et al. (1993). Removal of cyanogenic glycosides of flaxseed. *Food Chemistry*, 48:263–266.

WHO (1985a). *Guidelines for the study of dietary intakes of chemical contaminants*. Geneva, Switzerland, World Health Organization.

WHO (1985b). *Energy and protein requirements. Report of a joint FAO/WHO/UNU expert consultation.* Geneva, Switzerland, World Health Organization (Technical Report Series, No. 724).

WHO (2006). *GEMS/Food consumption cluster diets*. Geneva, Switzerland, World Health Organization, Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (http://www.who.int/foodsafety/chem/gems/en/index1.html; accessed January 2011).

WHO (2008). *Highest reported 97.5th percentile consumption figures (eaters only) for various commodities by the general population and children ages 6 and under (updated April 2008).* Geneva, Switzerland, World Health Organization.

WHO (2009a). *Cyanide in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality*. Geneva, Switzerland, World Health Organization (WHO/HSE/WSH/09.01/3).

WHO (2009b). *Cyanogen chloride in drinking-water. Background document for development of WHO Guidelines for Drinking-water Quality.* Geneva, Switzerland, World Health Organization (WHO/HSE/WSH/09.01/9).

WHO (2011a). *Acute and chronic dietary exposure assessments: international estimated short-term intake (IESTI) and international estimated daily intake (IEDI).* Geneva, Switzerland, World Health Organization (http://www.who.int/foodsafety/chem/acute_data/en/index1.html; accessed 1 April 2011).

WHO (2011b). *Guidelines for drinking-water quality*, 4th ed. Geneva, Switzerland, World Health Organization.

WHO Multicentre Growth Reference Study Group (2006). *WHO child growth standards—Length/height-for-age, weight-for-age, weight-for-length, weight-for-height and body mass index-for-age: methods and development.* Geneva, Switzerland, World Health Organization (http://www.who.int/childgrowth/standards/technical_report/en/index.html).

Willhite CC (1982). Congenital malformations induced by laetrile. *Science*, 215(4539):1513–1515.

Willhite CC, Smith RP (1981). The role of cyanide liberation in the acute toxicity of aliphatic nitriles. *Toxicology and Applied Pharmacology*, 59(3):589–560.

Yeoh HH, Egan SV (1997). An enzyme-based dip-stick for the estimation of cyanogenic potential of cassava flour. *Food Chemistry*, 60:119–122.

Yeoh HH, Sun F (2001). Assessing cyanogen content in cassava-based food using the enzyme-dipstick method. *Food and Chemical Toxicology*, 39:649–653.

Yessoufoua A et al. (2006). Cassava-enriched diet is not diabetogenic rather it aggravates diabetes in rats. *Fundamental & Clinical Pharmacology*, 20:579–586.

Zagrobelny M et al. (2004). Cyanogenic glucosides and plant–insect interactions. *Phytochemistry*, 65:293–306.

FUMONISINS (addendum)

First draft prepared by

A.S. Bulder,[1] D. Arcella,[2] M. Bolger,[3] C. Carrington,[3] K. Kpodo,[4] S. Resnik,[5] R.T. Riley,[6] G. Wolterink[1] and F. Wu[7]

[1] Centre for Substances and Integrated Risk Assessment, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
[2] European Food Safety Authority, Parma, Italy
[3] Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, Maryland, United States of America (USA)
[4] Food Chemistry Division, Food Research Institute, Council for Scientific and Industrial Research, Accra, Ghana
[5] Facultad de Ciencias Exactas y Naturales, Ciudad Universitaria, Buenos Aires, Argentina
[6] Agricultural Research Service, United States Department of Agriculture, Athens, Georgia, USA
[7] Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pennsylvania, USA

1. EXPLANATION

Fumonisins were previously evaluated by the fifty-sixth meeting of the Committee (Annex 1, reference *152*). The Committee established a group provisional maximum tolerable daily intake (PMTDI) for fumonisins B_1 (FB_1), B_2 (FB_2) and B_3 (FB_3), alone or in combination, of 2 µg/kg body weight (bw) on the basis of the no-observed-adverse-effect level (NOAEL) of 0.2 mg/kg bw per day for kidney toxicity and application of an uncertainty factor of 100. All of the estimates of exposure to FB_1 based on the available data on national consumption were well below the group PMTDI, even when exposure estimates for FB_1 were increased by 40% to account for the presence of FB_2 and FB_3. For the current evaluation, the Committee reviewed all relevant studies performed on fumonisins since 2001.

The B series of the fumonisins, including FB_1 (Chemical Abstracts Service [CAS] No. 116355-83-0), FB_2 (CAS No. 116355-84-1), FB_3 (CAS No. 136379-59-4) and FB_4 (CAS No. 136379-60-7), the major forms found in food, was described previously by the Committee. Since then, the number of known fumonisin analogues has greatly increased. The analogues can be classified into four main groups, A, B, C and P, which contain two tricarballylic acid moieties. Members of the series FBX are different from these, because they are esterified by other carboxylic acids, such as *cis*-aconitic acid, oxalylsuccinic acid and oxalylfumaric acid. New fumonisins recently described have their 19- or 20-carbon aminopolyhydroxyalkyl chain esterified by fatty acids, such as palmitic acid, linoleic acid and oleic acid (estimated as 0.1% of the FB_1 concentration).

The FB_1 toxin has 10 chiral centres; theoretically, therefore, 1024 stereoisomers can be produced. From culture material, currently 28 FB_1 isomers have been isolated and characterized (2.8% of the possible fumonisins). The identification and absolute configuration of the stereoisomers 3-*epi*-FB_3 and 3-*epi*-FB_4 have been elucidated since the previous evaluation. The hydrolysis of the tricarballylic esters at C-14 and C-15 gives rise to partially hydrolysed fumonisin B (PHFB) or totally hydrolysed fumonisin B (HFB) in food.

Fusarium verticillioides and *F. proliferatum* are the main sources of fumonisins in maize, and both can produce series B and C analogues. *Fusarium proliferatum* can also produce series P analogues.

Recent occurrence data have shown that *Aspergillus niger*, a common fungus growing on grapes, green coffee beans, onions, mango, corn and other cereals, peanuts and dried fruits, is able to produce FB_1, FB_2, FB_4 and FB_6. Some strains of *Fusarium oxysporum*, a worldwide fungal contaminant, also produce fumonisin C analogues.

The term "hidden fumonisin" is used only for non-covalently bound derivatives, which are formed through an interaction between fumonisins and matrix macroconstituents or physical entrapment. "Bound fumonisin" refers only to those compounds that involve covalent linkages between the analyte and the matrix constituents. The linkages may involve the fumonisin free amino group or the carboxylic moieties. A few studies conducted with a limited number of samples show that substantial amounts of hidden or bound fumonisins may be present in raw maize, and commonly used analytical methods are not able to detect their occurrence.

2. BIOLOGICAL DATA

As in previous World Health Organization (WHO) evaluations (Annex 1, reference *153*; IPCS, 2000; IARC, 2002), the focus of this review will be FB_1. However, many useful studies have been conducted using fungal culture material (primarily *Fusarium verticillioides*, formerly *F. moniliforme*), partially purified preparations prepared from fungal culture material or naturally contaminated maize, all of which are likely to contain FB_2 and FB_3 and other fumonisins (Rheeder, Marasas & Vismer, 2002; Bartók et al., 2006) and other *Fusarium* metabolites that could contribute to the observed effects in animals. Nonetheless, studies with these more complex materials provide valuable insights into the toxic potential of maize infected with fumonisin-producing strains of *Fusarium*, which is consumed in large quantities by farm animals and humans in areas where maize is a dietary staple.

The term "fumonisins" will be used when the diets are likely to contain multiple fumonisins (FB_1, FB_2, FB_3, etc.). For some studies, only FB_1 levels in the diets are reported; typically, however, in naturally contaminated corn-based foods and *F. verticillioides* corn culture material, the amount of FB_2 plus FB_3 is about one third of the total ($FB_1 + FB_2 + FB_3$). There are some *Fusarium* strains that produce predominantly FB_2 or FB_3, and recently *Aspergillus niger* and some other Aspergilli have been shown to produce a variety of fumonisins on commodities other than corn (Varga et al., 2010). Only those studies are included for which an analysis of the fumonisin content was performed.

2.1 Biochemical aspects

This section briefly summarizes the current understanding of the various biochemical aspects covered and presents advances in the knowledge base since the fifty-sixth meeting of the Committee (Annex 1, reference *153*).

2.1.1 Absorption, distribution and elimination

The information summarized in the previous evaluation by the Committee on the absorption, distribution and elimination of FB_1 remains valid as of this review. Briefly, absorption, distribution and elimination are rapid, although very little is absorbed. There is some evidence that fumonisins can be partially metabolized in the gut, but metabolism by liver or other tissues has not been convincingly demonstrated (Annex 1, reference *153*; IPCS, 2000; IARC, 2002; Shephard, Van

der Westhuizen & Sewram, 2007; Voss, Smith & Haschek, 2007). Information on the transfer of fumonisins from feed to animal products is described in section 7.

(a) Absorption

In all animals studied, including pigs, laying hens, turkey poults, ducks and dairy cows, fumonisins are rapidly absorbed, and the quantity of FB_1 detected in plasma and tissues after oral administration is very low, indicating that absorption, while rapid, is very poor (negligible to <4% of the dose) (Annex 1, reference *153*; IPCS, 2000; IARC, 2002; Fodor et al., 2008; Tardieu et al., 2008, 2009; Dilkin et al., 2010). The bioavailability of FB_2 may be less than that of FB_1. Feeding studies have shown that in liver and kidney of animals fed diets containing FB_1, FB_2 and FB_3, FB_2 and FB_3 are accumulated less than expected based on the relative amounts in the feed (Riley & Voss, 2006; Fodor et al., 2008; Gazzotti et al., 2011).

In a cultured intestinal epithelial cell model, HFB_1 but not FB_1 was found to cross the monolayer (primarily from basolateral to apical), suggesting a carrier-mediated efflux process (De Angelis et al., 2005). Efflux of FB_1 from renal epithelial cells was shown to be much more rapid than uptake into the cells, also suggesting a possible efflux mechanism (Enongene et al., 2002). There is some evidence that FB_1 can interact with rat and human organic anion transporters (Tachampa et al., 2008).

(b) Distribution

Although fumonisins are distributed to most tissues, liver and kidney retain the highest concentrations of the absorbed material in all animals studied (Annex 1, reference *153*; reviewed in Voss, Smith & Haschek, 2007). FB_1 persists in kidney much longer than in plasma or liver; in male Sprague-Dawley rats (Riley & Voss, 2006) and Wistar rats (Annex 1, reference *153*; IPCS, 2000), the levels of FB_1 in kidney can be 10 times the levels in liver. Whereas radiolabelled fumonisin has been detected in brains of pigs, little or no fumonisin has been detected in brain tissue of horses, although brain is a known target organ (Haschek, Voss & Beasley, 2002). Until recently, it was believed that fumonisins could not cross the placenta and enter the developing embryo (Annex 1, reference *153*); however, recent studies in mice have detected ^{14}C-labelled FB_1 in embryos and placenta after intraperitoneal administration to pregnant dams on gestation day 10.5 (unpublished results cited in Gelineau-Van Waes et al., 2005), an observation confirmed by the presence of elevated levels of free sphinganine, a biomarker for fumonisin inhibition of ceramide synthase in fetuses of maternally exposed mice (Gelineau-Van Waes et al., 2005).

(c) Elimination

When FB_1 is dosed intraperitoneally or intravenously, initial elimination from tissues is rapid, with no evidence of metabolism, but extensive enterohepatic circulation occurs (Annex 1, reference *153*; IPCS, 2000; Shephard, Van der Westhuizen & Sewram, 2007; Voss, Smith & Haschek, 2007). Several studies using different routes of exposure and different animal species have shown that fumonisins are excreted primarily in the faeces either unchanged or with the loss of one or both of the tricarballylic acid side-chains. Low levels of FB_1 can be detected

in the urine of animals exposed experimentally to fumonisin, including rabbits (Orsi et al., 2009), rats (Cai, Tang & Wang, 2007), pigs (Fodor et al., 2008; Dilkin et al., 2010), horses (Tumbleson et al., 2003) and vervet monkeys (Shephard, Van der Westhuizen & Sewram, 2007). In pigs, less than 1% of the oral dose is recovered in urine (Annex 1, reference *153*; IPCS, 2000; Dilkin et al., 2010).

Studies in humans also indicate that very little fumonisin is excreted in urine (Van der Westhuisen et al., 2011a), predominantly FB_1, and that the urinary half-life is short (48 hours) (Riley, 2010).

It has been estimated that in pigs exposed to dietary FB_1 at 2–3 mg/kg bw, a withdrawal period of at least 2 weeks would be required for the FB_1 to be eliminated from the liver and kidney (Annex 1, reference *153*; IPCS, 2000). Several studies have confirmed this finding using the persistence of free sphinganine as a biomarker in kidney and liver to show that although FB_1 is rapidly eliminated, the elevation of the biomarker persists much longer (Annex 1, reference *153*; Enongene et al., 2002). In male Wistar rats receiving a single gavage dose of FB_1, urinary sphinganine increased for 72 hours before returning to control levels at 96 hours (Direito et al., 2009). Although the half-life of FB_1 after oral dosing is not known, it is probably between 8 and 48 hours, based on what is known from the parenteral route, the time required to reach peak levels in plasma (1–7 hours) after gavage and the estimated time for complete clearance from liver and kidney (2 weeks) (Annex 1, reference *153*; IPCS, 2000).

2.1.2 Biotransformation

Microbial metabolism most likely occurs in the gut of monogastric animals, as $PHFB_1$ (lacking one tricarballylic acid side-chain) and, to a lesser extent, HFB_1 (lacking both side-chains) were recovered in the faeces but not in the bile of vervet monkeys (Annex 1, reference *153*). Most (60–90%) of the total FB_1 found in ruminant faeces was present as the partially hydrolysed form. In non-ruminants, the parent compound was the dominant species present (IPCS, 2000). However, studies in pigs have reported significant amounts of HFB_1 and $PHFB_1$ in faeces and tissues (Fodor et al., 2008). Whether the HFB_1 was produced in the tissues was not determined.

Fumonisins do not appear to be metabolized in vitro or in vivo by animal tissues (Annex 1, reference *153*), even though they are clearly excreted in bile, and HFB_1 and $PHFB_1$ have been reported in tissues (Fodor et al., 2008). One study suggested that a P450 isoform is capable of producing an FB_1 metabolite (Marvasi et al., 2006). Nonetheless, no convincing evidence has been reported of in vivo or in vitro metabolism by cytochrome P450, microsomal esterase or any other microsomal enzyme (Annex 1, reference *153*). However, studies have shown that P450 activity can be altered as a result of the inhibition of the enzyme ceramide synthase by fumonisin (Annex 1, reference *153*).

2.1.3 Effects on enzymes and other biochemical parameters relevant to the mode of action

The mechanisms of action, as described in detail in previous evaluations by the Committee and others (see Table 1 in Annex 1, reference *153*; Figure 1 in IPCS,

2000; Figures 3, 4, 5 and 6 in IARC, 2002), continue to be supported by many recent studies. The most likely proximate cause (key event) setting into motion the biochemical and molecular events leading to the toxicity and other effects of fumonisins is disruption of lipid metabolism as a consequence of inhibition of ceramide synthases (sphingoid base *N*-acyltransferases), key enzymes in the de novo sphingolipid biosynthesis pathway (Annex 1, reference *153*; IPCS, 2000; IARC, 2002). The ability of fumonisin to interact with ceramide synthases is due to its close structural similarity to sphingoid bases, most notably the recently discovered 1-deoxysphinganine, which, like fumonisin, lacks a hydroxyl group on C-1 and is found in large quantities in liver of mice fed diets containing FB_1 (Zitomer et al., 2009).

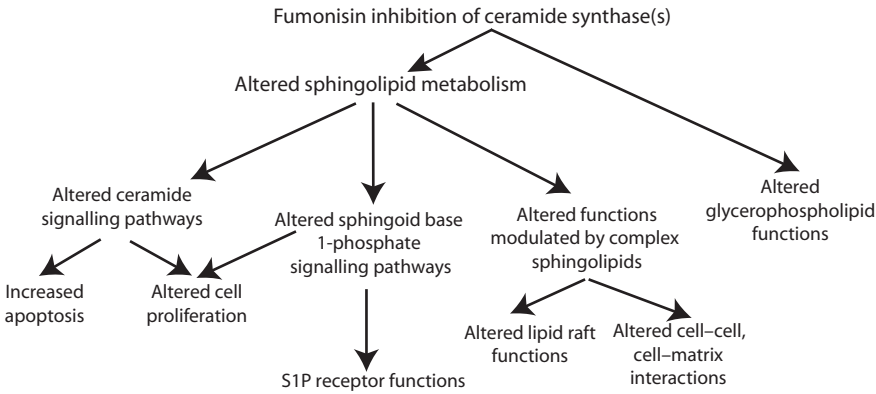
The major biochemical and cellular consequences of fumonisin inhibition of ceramide synthases are the accumulation of free sphingoid bases (Van der Westhuizen et al., 2004) and sphingoid base 1-phosphates (Kim et al., 2006, 2007; Riley & Voss, 2006; Cai, Tang & Wang, 2007; Burns et al., 2008; Voss et al., 2009, 2011), depletion of more complex sphingolipids (Howard et al., 2002; Voss et al., 2009) and the global disruption of lipid metabolism (Annex 1, reference *153*; IPCS, 2000; Gelderblom et al., 2002b; IARC, 2002; Burger et al., 2007; Raa et al., 2009).

The disruption of sphingolipid and glycerophospholipid metabolism is closely correlated with the extent and severity of the fumonisin-induced animal diseases, including tumour promotion (Carlson et al., 2001; Gelderblom & Marasas, 2011), carcinogenicity (NTP, 2001) and neural tube defects (NTDs) (Gelineau-Van Waes et al., 2005). The dose-dependent changes in sphingolipids (Riley & Voss, 2006; Voss et al., 2011) and glycerophospholipid and fatty acid composition of cell membranes (Gelderblom et al., 2002b; Van der Westhuizen et al., 2004; Burger et al., 2007) are consistent with the disruption of lipid metabolism as the underlying basis for fumonisin toxicity.

Evidence for in vivo fumonisin inhibition of ceramide synthase using the accumulation of sphingoid bases and sphingoid base 1-phosphates and depletion of more complex sphingolipids as early indicators of potential fumonisin toxicity in animals has been used successfully to demonstrate fumonisin exposure, structure–activity relationships, tissue specificity, strain/sex susceptibility and the efficacy of intervention strategies in poultry, fish, rats, mice, pigs and horses (Annex 1, reference *153*; IPCS, 2000; Howard et al., 2002; IARC, 2002; Smith et al., 2002; Fernandez-Surumay et al., 2005; Piva et al., 2005; Tran et al., 2005; Collins et al., 2006; Riley & Voss, 2006; Voss, Gelineau-Van Waes & Riley, 2006; Burns et al., 2008; Voss et al., 2009, 2011).

The fumonisin-induced disruption of lipid metabolism and consequent alterations in lipid composition of cell membranes and the changes in intracellular and extracellular concentrations of lipid mediators alter the expression and activity of signalling and regulatory pathways that control physiological processes critical for cell growth, differentiation and normal cell function (Annex 1, reference *153*; Voss et al., 2006a). The specific biochemical aspects of fumonisin disruption of sphingolipid metabolism that are most likely to alter cell regulation, leading to increased cell death and altered cell proliferation, are 1) increased free sphingoid bases and their 1-phosphates, 2) alterations in complex sphingolipids and 3) decreased ceramide

Figure 1. Biochemical and cellular consequences of fumonisin inhibition of ceramide synthase(s) and global disruption of lipid metabolism



S1P = sphingoid base 1-phosphate

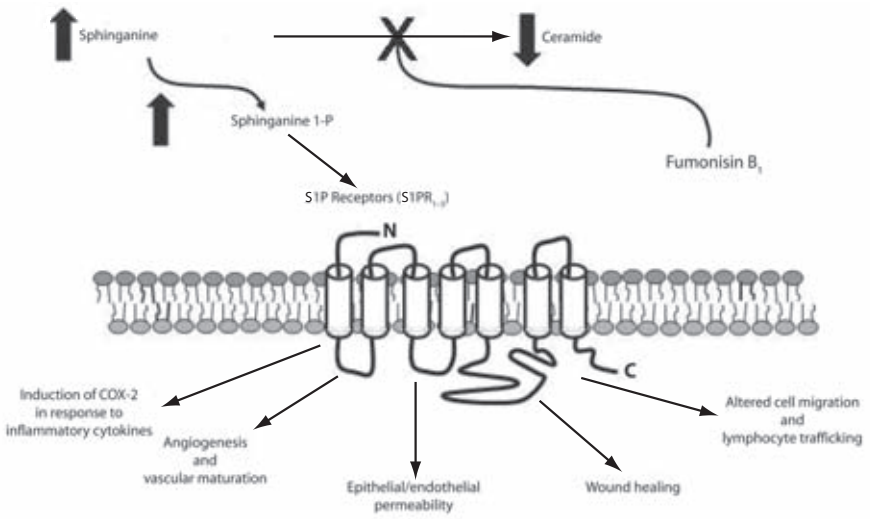
and ceramide 1-phosphate biosynthesis (Figure 1). Free sphingoid bases and ceramide can induce cell death; thus, fumonisin inhibition of ceramide synthase can inhibit cell death and other cellular processes dependent on ceramide generated de novo. Conversely, redirection of sphinganine into sphinganine 1-phosphate is a potential survival signal stimulating cell proliferation. For this reason, fumonisin has been used in several hundred short-term studies since 2001 to demonstrate the role of sphingosine 1-phosphate, ceramide and more complex sphingolipid biosynthesis in processes that control diverse cellular responses; for examples, see Table 4 in IPCS (2000) and Table 3 in reference *153* in Annex 1.

A decrease in pools of more complex sphingolipids will disrupt cell–cell and cell–matrix interactions and alter vitamin uptake and other processes mediated via glycosylphosphatidylinositol-anchored proteins associated with lipid rafts (Stevens & Tang, 1997; Decker & ffrench-Constant, 2004; Gelineau-Van Waes et al., 2005; Abdel Nour et al., 2007) and endosome-mediated retrograde transport of molecules to the Golgi apparatus (Raa et al., 2009).

Conversely, more prolonged inhibition of ceramide synthase results in accumulation of high intracellular concentrations of free sphingoid bases, which promotes free sphingoid base–induced cell death (Annex 1, reference *153*; IPCS, 2000; IARC, 2002). In mouse liver, in addition to sphinganine, large amounts of 1-deoxysphinganine accumulate, with only small amounts of sphingoid base 1-phosphates (Zitomer et al., 2009; Voss et al., 2009), whereas in rat kidney, there is no detectable 1-deoxysphinganine, but large amounts of sphinganine 1-phosphate (Riley & Voss, 2006; Voss et al., 2011).

Fumonisin-induced elevation in sphingoid base 1-phosphates in tissues and blood can disrupt signalling associated with extracellular receptors (sphingoid base 1-phosphate receptors). Sphingoid base 1-phosphate receptors ($S1PR_{1–5}$) regulate

Figure 2. Sphingoid base 1-phosphate receptor (S1PR) functions are potentially modulated by fumonisin-induced elevation in sphinganine 1-phosphate (modified from Eaton et al., 2011)



vascular physiology and other physiological processes that are critical for immune response and developmental processes (Chalfant & Spiegel, 2005; Gelineau-Van Waes et al., 2009) (Figure 2). Disruption of many of these processes is likely to be involved in the adverse effects of fumonisin seen in pigs and horses (see section 2.2).

The kinetics of the biosynthesis and degradation of the various bioactive and structurally critical sphingolipid pools and alterations in glycerophospholipids in tissues will be important factors in the observed downstream effects and ultimately the toxicity and carcinogenicity of fumonisins. Indirect evidence for the key role that disrupted sphingolipid metabolism plays in fumonisin tumorigenicity is the recent discovery that mice deficient in ceramide synthase spontaneously develop liver tumours (Imgrund et al., 2009; Pewzner-Jung et al., 2010a,b).

2.2 Toxicological studies

Since the last evaluation, a large number of fumonisin toxicity studies have been published. Studies were included in this monograph addendum based on the following criteria:

- studies using pure or purified fumonisins;
- studies using naturally contaminated feed or *F. verticillioides* culture material for which the fumonisin content was determined;
- specific in vitro studies aimed at elucidating new mechanistic aspects of fumonisin toxicity;
- mechanistic studies using FB_1 as a model compound for ceramide synthase inhibition;

- studies using chickens, ducks or turkeys as test species if they elucidated mechanistic aspects of fumonisin toxicity relevant to humans.

This section summarizes toxicological studies conducted since the 2001 evaluation for the purpose of revealing the acute, short-term and long-term toxicity of animal exposure under controlled conditions to FB_1, FB_2 and FB_3, individually or in combination. As was true for the previous evaluation, there have been a large number of studies conducted using diets prepared using *F. verticillioides* culture material known to contain a mixture of fumonisins, primarily FB_1, FB_2 and FB_3, and possibly other mycotoxins. There have also been in vivo studies conducted with pure FB_1, but fewer than were available at the time of the previous evaluation. The focus of this section will be studies conducted in animal models to reveal dose–response relationships or to reveal novel aspects of fumonisin toxicity that serve to expand the understanding of the biochemical basis for fumonisin toxicity and carcinogenicity and its potential for contributing to animal and human disease.

As was stated in the previous evaluation and confirmed in more recent studies, FB_1 is sex, strain and species specific in terms of the target organs and susceptibility. Liver and kidney are target organs in all species, and in both liver and kidney, evidence of disruption of lipid metabolism and increased apoptosis, oncotic necrosis and regeneration are early signs of fumonisin toxicity. In the previous evaluation, it was noted that nutritional factors could play an important role in modulating fumonisin toxicity. Several studies have identified nutritional factors that modulate fumonisin toxicity, such as folate and antioxidant status. Many more studies since 2001 confirm that antioxidants protect against both FB_1-induced tissue damage and damage induced by exposure to diets or treatments that contain fumonisins. It has been hypothesized (Gelderblom et al., 2004; Gelderblom & Marasas, 2011) that the high-protein diet used in the United States National Toxicology Program's (NTP) long-term carcinogenicity study in Fischer 344N rats (NTP, 2001) could have contributed to the development of kidney tumours in the absence of liver tumours in male rats rather than liver tumours in the absence of kidney tumours, as was seen in male BD IX rats (Gelderblom et al., 1991). High-protein diets are well known to sensitize rat kidney to chronic progressive nephropathy (Hard & Seely, 2005). Likewise, choline, methionine, B vitamins and folate-deficient diets have been proposed to sensitize rat liver to toxicity (reviewed in Gelderblom et al., 2004).

2.2.1 Acute toxicity

Acute toxicity studies with FB_1 are summarized in Table 1.

As in the previous evaluation, there are no studies showing lethality from a single dose of pure FB_1. However, subacute repeated dosing of the combination of myriocin (1 mg/kg bw intraperitoneal) and FB_1 (2.25 mg/kg bw subcutaneous) for 5 days was lethal to BALB/c mice (preliminary results mentioned in He, Riley & Sharma, 2005), even though myriocin alone caused no signs of toxicity.

Since 2001, there have been several single-dose studies demonstrating that high doses can induce biochemical alterations and other changes indicative of

Table 1. Overview of acute toxicity studies with fumonisin B_1

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw)[a]	Route	Effect	LOAEL (mg/kg bw)[a]	NOAEL (mg/kg bw)[a]	Reference
Female BALB/c mice, 6 weeks old, about 22 g	FB_1 (>98%)	5 days	5	—	0 or 2.25	Subcutaneous	Mortality (combined treatment with myriocin, but myriocin alone did not induce mortality)	—	—	He, Riley & Sharma (2005) (cited preliminary results)
Male BALB/c mice, 7 weeks old, about 20 g	Purified FB_1 (>90%)	Single dose	4	—	0 or 25	Gavage	Induction of genes involved in cytokine signalling pathways 4–8 h post-treatment	25	<25	Bhandari & Sharma (2002)
Male Swiss NIH mice, 8 weeks old	Purified FB_1 (>95%)	Single dose	4	—	25 (zero time point as control)	Gavage	Elevated free sphinganine in liver 2 h post-treatment	25	<25	Enongene et al. (2002)
Male Fischer 344 rats, 90–110 g	FB_1 (>98%)	Single dose, 7 days observation	5	—	0, 1.0, 2.15, 4.64, 10.0, 21.5 or 46.4	Gavage	Reduced feed intake, signs of stress	21.5	10.0	McKean et al. (2006)
							Lethality	—	46.4	
Male Fischer 344 rats, 5 weeks old, 100 ± 20 g	Purified FB_1 (~98%)	Single dose, 10 days observation	3–6	—	0, 10 or 25	Gavage	Elevation in the urinary sphinganine to sphingosine and sphinganine 1-phosphate to sphingosine 1-phosphate ratios	10	<10	Cai, Tang & Wang (2007)
Male Wistar rats, ~230 g	Purified FB_1 (98%)	Single dose	6	—	0, 5, 50 or 500 µg/kg bw	Gavage	Necrotic cells in liver	5 µg/kg bw	<5 µg/kg bw	Domijan et al. (2008)
							Increased number of apoptotic cells in liver	50 µg/kg bw	5 µg/kg bw	
Male weaned piglets, 8 weeks old, 25 kg	FB_1 (Sigma)	Single dose	4	—	5 (no control group)	Gavage	Behavioural and clinical signs of toxicity suggestive of onset of pulmonary oedema	5	<5	Dilkin et al. (2010)

LOAEL, lowest-observed-adverse-effect level; NOAEL, no-observed-adverse-effect level

[a] Unless otherwise specified.

toxicity. The following are some of the single-dose effects of pure/purified (>90%) FB_1 observed in mice, rats and pigs.

(a) Mice

In male BALB/c mice, a single oral FB_1 dose of 25 mg/kg bw, which was shown in an earlier study to induce signs of liver toxicity, caused the induction of genes involved in cytokine signalling pathways 4–8 hours post-treatment (Bhandari & Sharma, 2002). In another study in Swiss NIH mice, the same dose significantly elevated free sphinganine in liver within 2 hours (Enongene et al., 2002).

(b) Rats

Based on a single gavage dose and observation for 7 days (McKean et al., 2006), it was concluded that whereas high doses of FB_1 caused reduced feed intake and observable signs of stress, the lethal oral single dose in male Fischer 344 rats was greater than 46.4 mg/kg bw, the highest dose administered. For comparison, aflatoxin B_1 (AFB_1) caused 100% mortality (5/5) at 96 hours after a single gavage administration with 4.6 mg/kg bw in the same study.

Also in male Fischer 344 rats, Cai, Tang & Wang (2007) showed that a single oral dose of 10 or 25 mg/kg bw caused significant elevation in the urinary sphinganine to sphingosine and sphinganine 1-phosphate to sphingosine 1-phosphate ratios compared with the control group, and the effect persisted until at least 10 days after dosing (the last sample time).

In male Wistar rats receiving a single gavage FB_1 dose of 0, 5, 50 or 500 µg/kg bw, significant increases in the number of apoptotic cells in liver were observed after 24 hours at 5 µg/kg bw (Domijan et al., 2008). At 50 and 500 µg/kg bw, the number of apoptotic cells in liver was significantly increased from 4 to 48 hours post-treatment, and necrotic cells were observed at all dose levels and times. Thus, the single-dose NOAEL for apoptosis in male Wistar rat liver would be below 5 µg/kg bw. In the opinion of the Committee, these findings are inexplicable when compared with the dosages that induce increased apoptosis in other studies in male rats (Annex 1, reference *153*; NTP, 2001) and were therefore not used in the evaluation.

(c) Pigs

Pigs given a single oral dose of 5 mg/kg bw did not develop pulmonary oedema but did show behavioural and clinical signs of toxicity suggestive of its onset; however, no treatment control group was tested (Dilkin et al., 2010). The Committee could not determine a NOAEL.

2.2.2 Short-term studies of toxicity

(a) Studies using purified fumonisin B_1

The short-term studies of the toxicity of pure or purified FB_1 are summarized in Table 2.

Table 2. Overview of short-term studies of toxicity using pure/purified fumonisin B_1

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Mice										
Female SV129 mice + their PPARα null counterparts	FB_1 (>98% purity or from culture material)	7 days	5	0 or 300	Equivalent to 0 or 45	Diet	Increased sphinganine concentration, apoptosis and cell proliferation in liver Similar effects with purified FB_1 and FB_1 from culture	45	<45	Voss et al. (2006a)
Female B6C3F1/Nctr BR mice (6 weeks old, average 16.4 g)	Purified FB_1 (>97%)	28 days	16 (control) or 8 (treated)	0, 10, 50 or 100 (target); 0, 10, 52 and 103 (actual)	Equal to 0, 2.2, 11.5 and 22.9	Diet	Increased sphinganine to sphingosine ratio (1.5 mg/kg bw per day)			Howard et al. (2002)
							Elevation in serum cholesterol, alkaline phosphatase, total bile acids; decreases in liver ceramide; increased centrilobular apoptosis in liver, hepatocellular hypertrophy, vacuolization, Kupffer cell hyperplasia, macrophage pigmentation	7.5	1.5 (1.6 using data from study)	
							Decrease in liver weight	15	7.5	

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Male wild-type and p53+/– (P53N5) mice derived from C57BL/6 mice (5–7 weeks old, average 27.4–29.7 g)	FB_1 (97%)	26 weeks	10	0, 5, 50 or 150	Equal to 0, 0.4, 4 or 12	Diet	Megalocytic hepatocytes	0.4	<0.4	Bondy et al. (2010)
							Increased apoptosis and necrosis (p53+/–), elevated sphinganine, sphinganine 1-phosphate and deoxysphinganine in liver and kidney (wild type and p53+/–)	4	0.4	
							Reduced final body weight (p53+/–), mild haematological changes (wild type and p53+/– mice), increased apoptosis and necrosis (wild type), nodular appearance of liver consisting of megalocytic hepatocytes (wild type and p53+/–)	12	4	
Rats										
Male Fischer 344 rats (age unknown)	FB_1 (90–95%)	21 days	3	0, 10, 25, 50, 100, 250 or 500	Equivalent to 0, 1, 2.5, 5, 10, 25 or 50	Diet	Altered fatty acid content in phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol	1	<1	Gelderblom et al. (2002b)
							Increased total phospholipids	5	2.5	

Table 2 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
							Decreased microsomal delta 6 desaturase activity	10	5	
							Reduced body weight gain, increased total cholesterol, increased phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol	25	10	
Male Fischer 344 rats (130–140 g)	FB_1 (92–95%)	14 days FB_1, 2-AAF (3 days) + PH 3 weeks later, sacrifice 14 days later (total: 53 days)	8–10	—	1.4, 4.2, 11.4, 21 or 35	Gavage	Reduced body weight (≥11.4 mg/kg bw per day) Degenerative changes in liver: increased apoptosis, oval cell proliferation and mitosis, enhanced induction of $GSTP^+$ foci (≥21 mg/kg bw per day) Increased lipid peroxidation (35 mg/kg bw per day) Remark: enhanced induction of $GSTP^+$ foci in all dose regimes except treatment with $PbNO_3$; not mentioned whether other effects were dependent on regime	11.4	4.2	Gelderblom et al. (2001)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
		14 days FB_1, single dose of CCl_4 or $PbNO_3$ after 7th gavage treatment, 2-AAF/PH 3 weeks later, sacrifice 14 days later	8–10	—	1.4, 4.2, 11.4 or 21	Gavage				
		14 days FB_1, PH after 7th gavage treatment, 2-AAF/ CCl_4 treatment 3 weeks after end of FB_1 dosing, sacrifice 14 days later	8–10	—	1.4, 4.2, 11.4 or 21	Gavage				

Table 2 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
		14 days vehicle solvent, PH at day 7, 2-AAF/CCl_4 treatment after 3 weeks, sacrifice 14 days later	8–10	—	0	Gavage				
Male Fischer 344 rats (7, 26 and 52 weeks old)	FB_1 (92–95%)	21 days	10	0 or 250	Equivalent to 0, 25 (7 weeks), 20 (26 weeks) or 15 (52 weeks), based on reported feed intakes	Diet	Reduced body weight gain, weight loss Reduced feed intake Liver: lipid accumulation, increased apoptosis, oval cell proliferation and early fibrosis, increased $GSTP^+$ foci	25 (7 weeks), 20 (26 weeks) or 15 (52 weeks)	<15	Gelderblom et al. (2008)
Male Wistar rats	FB_1 (97%)	2 or 7 days	5	—	Equivalent to 5, assuming 10% oral absorption (0.5 intraperitoneal)	Intraperitoneal	Liver: increased glutathione levels and Hsp70 expression Kidney: decreased levels of glutathione and Hsp70 expression	0.5 (intraperitoneal)	<0.5 (intraperitoneal)	Rumora et al. (2007)

2-AAF, 2-acetylaminofluorene; GSTP, placental glutathione *S*-transferase; PH, partial hepatectomy; PPARα, peroxisome proliferator-activated receptor alpha

(i) Mice

Female SV129 mice and their peroxisome proliferator-activated receptor alpha (PPARα) null counterparts (five animals per treatment group) were fed diets containing FB_1 concentrations of 0 or 300 mg/kg diet (equivalent to 0 or 45 mg/kg bw per day) prepared with either purified FB_1 (>98% purity) or *F. verticillioides* (MRC826) culture material for 7 days. Other groups were also fed the peroxisome proliferator WY-14,643 (500 mg/kg diet). The purpose of the study was to determine if FB_1 caused peroxisome proliferation in mice and to compare the effects of pure FB_1 with those of *F. verticillioides* culture material on genes that control cell proliferation. The results showed that neither pure FB_1 nor *F. verticillioides* culture material induced peroxisome proliferation. The histopathological and sphingolipid effects found in the mice given FB_1 or culture material were qualitatively (target organs, type of findings) and quantitatively (degree of severity) similar. Furthermore, the gene expression profiles for genes regulating cell proliferation, signal transduction and glutathione metabolism were similar (62% similarity) for FB_1 and culture material (Voss et al., 2006a). As only one dose was tested, a NOAEL could not be determined by the Committee, but would be below 45 mg/kg bw per day.

Female B6C3F1/Nctr BR mice (6 weeks old, 16 animals in the control group and 8 in the treatment group) were fed diets containing a low, medium and high level of purified (>97%) fumonisin—FB_1, FB_2, FB_3, HFB_1, *N*-(acetyl) FB_1, FP_1 or *N*-(carboxymethyl) FB_1—for 28 days. The target levels were 0, 14, 70 and 140 µmol of fumonisin derivative per kilogram diet (equal to 0, 10, 50 and 100 mg equivalents of FB_1 per kilogram diet). Concentrations were expressed in molar units because of the different molecular weights of the derivatives. The stability of the fumonisins in the diets was confirmed at the end of the study. None of the treatment groups showed any difference in average weight gain (0.58 g per mouse per week) or feed consumption (3.9 g per mouse per day) compared with the control group. The only fumonisin derivative to cause any toxicity was FB_1, which caused significant elevation in serum cholesterol, alkaline phosphatase and total bile acids and significant decreases in liver ceramide at the medium and high dose levels (72 and 143 µmol of FB_1 per kilogram diet based on analysis of the diet and calculated using the authors' data to be equal to 11.5 and 22.9 mg/kg bw per day) and a significant decrease in liver weight at the high dose level. The liver weight was also decreased at the medium dose level, but not significantly. The sphinganine to sphingosine ratio was significantly increased at all dose levels, including the low dose (14 µmol of FB_1 per kilogram diet, equal to 2.2 mg/kg bw per day). Based on the histological examination, only liver was affected, and the primary effect was increased centrilobular apoptosis (ApoTag) in only the medium- and high-dose livers. The rate of apoptosis was 1.8, 1.8, 7.9 and 17.5 per 2000 hepatocytes in the control, low-dose, medium-dose and high-dose groups. Other changes observed only in the medium- and high-dose groups were hepatocellular hypertrophy and vacuolization, Kupffer cell hyperplasia and macrophage pigmentation. It was concluded by the authors that as only FB_1 caused hepatotoxicity and as FB_1 hepatotoxicity correlates with hepatocarcinogenicity, the human risk assessment can be based solely on FB_1 content (Howard et al., 2002). The overall lowest-observed-adverse-effect level (LOAEL) was 72 µmol of FB_1 per kilogram diet (52 mg/kg diet) for centrilobular apoptosis, which was calculated using the authors' data to be equal to 11.5 mg/kg

bw per day. The NOAEL was 2.2 mg/kg bw per day. The Committee concluded that the NOAELs and LOAELs found were consistent with those reported previously in mice (Annex 1, reference *153*; NTP, 2001).

Male transgenic p53+/– and corresponding wild-type mice (10 animals per group, 5–7 weeks old, initial starting weights in the dose groups 27.4 ± 2.9 g to 29.7 ± 3.4 g) were fed diets (AIN 76) containing FB_1 (97%) at levels of 0, 5, 50 or 150 mg/kg diet for 26 weeks. During the study, mice received water and control or test diet ad libitum. Body weight and feed consumption were measured weekly. Based on the mean body weight over the entire 26-week exposure period, the FB_1 exposure for the transgenic/wild-type mice was 0/0, 0.39/0.37, 3.87/3.88 and 12.2/12.6 mg/kg bw per day in the animals fed the 0, 5, 50 and 150 mg/kg diets, respectively.

After 26 weeks, final body weights were recorded, and all mice were exsanguinated by cardiac puncture under isoflurane anaesthesia. At necropsy, a gross visual examination was performed on each mouse, and organ weights were recorded for liver, kidney, thymus and spleen. Liver, right kidney, thymus, spleen, oesophagus, trachea, thyroid, stomach (including forestomach), ileum (including Peyer's patches), mesenteric lymph nodes, popliteal lymph nodes, heart and lungs were fixed for histopathological examination. Apoptosis, oncotic necrosis in tissues and proliferative liver lesions were classified using light microscopy with a defined scoring scheme. Whole blood was collected in ethylenediaminetetraacetic acid at necropsy for haematology. The following parameters were measured: red blood cells, haemoglobin, haematocrit, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, red cell distribution width, platelets, mean platelet volume, white blood cells, neutrophils, lymphocytes, monocytes, eosinophils and basophils. Immunophenotyping in whole blood and splenocyte suspensions was performed by flow cytometry using antibodies. Immunoglobulin A (IgA), IgG and IgM were determined in whole blood samples by sandwich enzyme-linked immunosorbent assay (ELISA). Sphingolipid analyses were performed on liver and kidney tissues.

There was no treatment-related mortality during the study, and no overt morbidity was observed in any dose group at necropsy. Mean final body weight was significantly lower in transgenic p53+/– mice consuming the high-dose diet compared with the corresponding transgenic p53+/– and wild-type controls; no significant differences in mean final body weight were observed in wild-type mice. There were no significant treatment-related changes in liver weight in wild-type or transgenic mice. Kidney weight (relative to body weight) was significantly elevated in transgenic mice at the high dose level, and spleen weights (relative to body weight) were elevated in wild-type and transgenic mice at the high dose level. Haematological changes in transgenic p53+/– and corresponding wild-type mice were mild and confined to the high-dose group. In both wild-type and transgenic p53+/– mice, there were no significant changes in liver pathology in the low-dose groups. Increased incidence of megalocytic hepatocytes was a dose-dependent effect observed at all dose levels above controls in both the wild-type and transgenic mice. In transgenic p53+/– mice, increased apoptosis and focal necrosis were statistically significant, relative to corresponding controls, in the medium- and high-dose groups. In wild-type mice, increased apoptosis was significant relative to corresponding controls in

the high-dose group, whereas increased focal necrosis was significant only in the medium-dose group. There were a number of significant changes in the severity of additional hepatic lesions in both wild-type and transgenic p53+/– mice, all in the high-dose groups. Livers from wild-type and transgenic p53+/– mice exposed to the high dose had a nodular appearance. Microscopic examination revealed more or less defined nodules of variable diameters consisting of megalocytic hepatocytes. Liver and kidney sphinganine, sphinganine 1-phosphate and deoxysphinganine levels were elevated in wild-type and transgenic p53+/– mice; changes were apparent or significant in the medium- and high-dose groups. The authors concluded that FB_1 was hepatotoxic to wild-type and transgenic p53+/– mice, and heterozygosity of the *p53* gene in transgenic p53+/– mice had a minimal or no effect on the toxicological outcomes associated with FB_1 exposure (Bondy et al., 2010). The Committee concluded that as effects were seen in the lowest dose group, a NOAEL could not be determined. Dose–response analysis was performed on this study in the evaluation.

(ii) Rats

Male Fischer 344 rats (three animals per group) were fed diets containing FB_1 (90–95% pure) at 0, 10, 25, 50, 100, 250 or 500 mg/kg diet for 21 days (equivalent to 0, 1, 2.5, 5, 10, 25 and 50 mg/kg bw per day). Body weight gain was significantly reduced in animals consuming 25 mg/kg bw per day for 21 days. The livers were homogenized, and the liver microsomes from all but the highest dose group were analysed for cholesterol, sphingomyelin and total phospholipids. The total phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylserine were determined, as was their fatty acid content. Total phospholipids increased in a dose-dependent manner, and the increase was statistically significant ($P < 0.05$) at 5 mg/kg bw per day; total cholesterol was increased at 25 mg/kg bw per day. The phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol levels also increased in a dose-dependent manner, and the differences were statistically significant ($P < 0.05$) at 25 mg/kg bw per day. The fatty acid contents of phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol were significantly altered in selected fatty acid pools even at the lowest dose level, with the most pronounced effects being seen in the fatty acid content of phosphatidylcholine and phosphatidylethanolamine at 10 mg/kg bw per day. The microsomal delta 6 desaturase activity was also significantly decreased at and above 100 mg/kg diet (Gelderblom et al., 2002b). The Committee could not determine a no-effect level for lipid metabolism, as effects on fatty acid content were apparent at the lowest dose level (equivalent to 1 mg/kg bw per day). In a previous study using a similar experimental design, the NOAEL for liver toxicity was greater than 1 mg/kg bw per day (Annex 1, reference *153*, Table 5). Thus, the effects on the fatty acid content of glycerophospholipids in liver microsomes occur at doses lower than those that cause overt liver toxicity.

Male Fischer 344 rats (8–10 animals per dose group) were gavaged with FB_1 (92–95%) daily at 1.4, 4.2, 11.4, 21 or 35 mg/kg bw for 14 days. Three animals from each dose group were killed after the 14-day fumonisin treatment, and the livers were analysed for thiobarbituric acid reactive substances (TBARS). Three weeks later, the remaining animals were given three treatments with a promoting stimulus

of 2-acetylaminofluorene (2-AAF) and partial hepatectomy, and the animals were sacrificed 14 days later (total study length = 53 days).

Two additional treatments were also part of the study. For the second treatment, animals (8–10 animals per dose group) were gavaged with FB_1 daily at 1.4, 4.2, 11.4 or 21 mg/kg bw for 14 days; after 7 days, the rats were dosed once with either carbon tetrachloride (CCl_4) or lead nitrate ($PbNO_3$), followed by the 2-AAF/partial hepatectomy treatment 3 weeks later and sacrifice 14 days later. In the third treatment, animals (8–10 per dose group) received a dosage regimen similar to the second group, but after seven gavage treatments, the rats were subjected to partial hepatectomy, followed by seven more gavage treatments with FB_1, a promoting stimulus of 2-AAF/CCl_4 3 weeks later and then sacrifice 14 days later.

A fourth group of animals (8–10 per dose group) received the solvent vehicle for 14 days with partial hepatectomy at day 7, the 2-AAF/CCl_4 treatment after 21 days and then sacrifice 14 days later.

The purpose of the various treatments was to investigate the roles of both lipid peroxidation and cell proliferation on the cancer-initiating potential of FB_1. FB_1 treatment significantly reduced body weight gain at and above 11.4 mg/kg bw per day. Evidence of increased lipid peroxidation (TBARS) was noted in the group receiving 35 mg/kg bw per day ($P < 0.05$). Degenerative changes, including increased apoptosis, oval cell proliferation and mitosis, were observed in the liver at and above 21 mg/kg bw per day. With the exception of the group treated with lead nitrate to stimulate hyperplasia, induction of placental glutathione *S*-transferase positive ($GSTP^+$) foci was noted in the high-dose groups (≥21 mg/kg bw per day) treated with FB_1 and the various treatments to stimulate regenerative cell proliferation (partial hepatectomy and carbon tetrachloride). It was concluded by the authors that regenerative cell proliferation sensitized the liver to FB_1-induced initiation (Gelderblom et al., 2001). The Committee concluded that, based on reduced body weight gain at and above 11.4 mg/kg bw per day, the NOAEL was 4.2 mg/kg bw per day, whereas for induction of $GSTP^+$ foci, it was 11.4 mg/kg bw per day, and for lipid peroxidation, it was 21 mg/kg bw per day.

Male Fischer 344 rats of different ages (7, 26 and 52 weeks old; 10 per age group) were fed diets containing FB_1 (92–95% pure) at 0 or 250 mg/kg for 21 days (equivalent to 0 or 25 mg/kg bw per day for the 7-week-old rats, 0 or 20 mg/kg bw per day for the 26-week-old rats and 0 and 15 mg/kg bw per day for the 52-week-old rats). Comparison of the 7-, 26- and 52-week-old rats fed FB_1 for 3 weeks showed that the body weight gain in all age groups was significantly reduced compared with their matched control groups; weight loss was recorded for the 26- and 52-week-old mice in the FB_1-treated groups. All three age groups showed lipid accumulation, increased apoptosis, oval cell proliferation and early fibrosis in the liver, but the effects were less severe in the 26- and 52-week-old animals. The 7-week-old rats also had significantly more $GSTP^+$ foci, indicating that younger rats are more susceptible to hepatotoxicity and induction of $GSTP^+$ foci, possibly due to reduced feed intake by the older animals. It was concluded by the authors that the low FB_1 exposure levels in the older rats are still sufficient to effect promotion and that FB_1-induced $GSTP^+$ foci are not due to the presence of spontaneous initiated cells in the liver noticed in older rats (Gelderblom et al., 2008). A NOAEL could

not be determined by the Committee, as all treatment groups showed either liver pathology or reduced weight gains, but would be lower than 15 mg/kg bw per day.

Male Wistar rats (five groups of five animals each) were intraperitoneally injected with FB_1 (97% pure) at 0 or 0.5 mg/kg bw per day for 2 or 7 days. The equivalent oral dose would be approximately 5 mg/kg bw per day, assuming 10% absorbance for an oral dose. Treatment-related effects were time and target organ dependent and included increased glutathione levels and Hsp70 expression in liver and decreased levels of glutathione and Hsp70 expression in kidney. It was concluded that kidney is more sensitive than liver to fumonisin-induced toxicity. A NOAEL could not be determined (Rumora et al., 2007).

Male Fischer 344 rats were fed diets containing 250 mg FB_1 per kilogram for 5 weeks (equivalent to 21 mg/kg bw per day) and then either 0 or 100 mg FB_1 per kilogram diet (7.5 mg/kg bw per day) for an additional 20 weeks followed by a return to the control diet (AIN 76) for an additional 25 weeks (total 50 weeks). The FB_1 was 92–95% pure. The modulating effects of 2-AAF on oval cell proliferation were compared. Treated groups contained more than six animals, and there were five control animals at the end of the study (50 weeks). Oval cell, $GSTP^+$ lesion area and fibrosis scores were significantly elevated compared with the controls. At the end of the study (50 weeks), postmortem examination revealed that one animal in the group fed FB_1 for 5 weeks with 250 mg FB_1 per kilogram and 20 weeks with 100 mg FB_1 per kilogram contained an unequivocal hepatocellular carcinoma. Hepatic adenomas were also noticed in the rats that received FB_1 for only 5 weeks and normal control diet for the remainder of the treatment period of 50 weeks, indicating that lesions induced by FB_1 during the first 5 weeks persisted throughout the study period. It was concluded by the authors that 2-AAF has a potentiating effect on the induction of cholangiofibromas induced by FB_1 (Lemmer et al., 2004). A NOAEL could not be determined by the Committee.

(b) Studies using diets containing fumonisin B_1 prepared from F. verticillioides *culture material*

The short-term studies of the toxicity of FB_1 prepared from *F. verticillioides* culture material are summarized in Table 3.

(i) Mice

Female BALB/c mice (24 animals per group) were fed diets prepared from *F. verticillioides* (MRC 826) culture material so as to contain 0, 50 or 150 mg FB_1 plus FB_2 per kilogram diet (based on high-performance liquid chromatography [HPLC], equivalent to 0, 7.5 or 22.5 mg/kg bw per day) for a total of 41 or 42 days. After 6 days on the test diets, 60 animals were inoculated (intraperitoneally) with 1000 bloodstream trypomastigotes of *Trypanosoma cruzi* (day 0 inoculated). "Satellite" control groups (four animals per group) that were fed the fumonisin diets but were never inoculated with *T. cruzi* were sacrificed on day 35 (total 41 days on fumonisin diets). The groups (five animals per time point) inoculated with *T. cruzi* were sampled on days 0, 14, 26 and 36. After 36 days (42 days on diets), there were minimal effects on growth, feed consumption or relative liver or kidney weights.

Table 3. Overview of short-term studies of toxicity using culture material

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Mice										
Female BALB/c mice (5–6 weeks old)	FB_1 (+ FB_2), from *F. verticillioides* culture material (MRC 826)	41–42 days	24	0, 50 or 150	0, 7.5 or 22.5	Diet	**FB_1 with or without inoculation of *Trypanosoma cruzi*:** Hepatocellular apoptosis and anisocytosis Increased liver sphinganine to sphingosine ratio **FB_1 with inoculation only:** Increased nitric oxide production by peritoneal macrophages Reduced numbers of trypomastigotes in the blood Decreased numbers of pseudocysts in the cardiac muscle	7.5	<7.5	Osborne et al. (2002)
Male TNFα-null and wild-type mice (12 ±1.5 weeks old)	FB_1 from culture material (*F. verticillioides*, MRC 826)	8 days	5	0 or 300–350	0 or 45–52	Diet	Null mice: increased hepatic apoptosis and mitotic figures, increased liver sphinganine	45	<45	Voss et al. (2006b)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Rats										
Male Sprague-Dawley rats (3–4 weeks old)	FB_1, FB_2 and FB_3 (at 1.00:0.45:0.10 by weight) from *F. verticillioides* (MRC 826) culture material	10 days	12	1.1 (control), 13.5 or 88.6 (total fumonisins)	0.11, 1.35 or 8.86 (total fumonisins)	Diet	Decreased absolute kidney weights, histological changes in both liver and kidney, increased sphinganine and sphinganine 1-phosphate	1.35 (total fumonisins)	0.11	Riley & Voss (2006)
Male Sprague-Dawley rats (73–74 g)	FB_1 from *F. verticillioides* M-2552	8 weeks	10	<0.1, <0.1 (controls), 0.3, 1.4, 2.9, 4.9, 9 or 25	Reported to be equal to <0.01, <0.01, 0.03, 0.10, 0.22, 0.35, 0.70 or 1.80	Diet	Authors: increased apoptotic pathology scores and ceramide synthase inhibition	*The Committee evaluated that this could be 0.10.*	*Committee: 0.03, but for the evaluation, BMD analysis was used on the results*	Voss et al. (2011)
							Decreased feed consumption, signs of kidney dysfunction, decreases in absolute and relative kidney weights	0.35	0..22	
Female mature Wistar rats (age unknown)	FB_1 from *F. verticillioides* culture material	35 days	13	0.2 (control), 10 or 20	Equivalent to 0.02, 0.82 or 1.98 using study data	Diet	Reduced final body weight and relative weight gain, reduced nutrient digestibility (≥0.82 mg/kg bw per day), increased feed conversion ratio (1.98 mg/kg bw per day)	0.82	0.02	Gbore (2010)

Table 3 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Rabbits										
Crossbred male rabbits (22–24 weeks old, 1.36 ± 0.01 kg)	Fumonisin from *F. verticillioides* MRC 826 (FB_1 + FB_2 + FB_3)	5 weeks	10	0.35 (control), 12.3 or 24.6	0.011, 0.37 or 0.74	Diet	Decrease in dry matter intake, increased feed conversion ratio	0.74	0.37	Ewuola et al. (2008)
Crossbred mature female rabbits (1.65–2 kg)	Fumonisin from *F. verticillioides* (MRC 826)	6 weeks	8	0, 5 or 10	0, 0.15 or 0.3	Diet	Weight loss, reduced dry matter intake, reduced haemoglobin, reduced lymphocytes, increased leukocytes, increased neutrophils, decreased total protein, increased ALT, AST and alkaline phosphatase	0.15	<0.15 *The Committee noted that there were numerous problems with the statistical analysis, and therefore this study was not used in the evaluation.*	Gbore & Akele (2010)
							Decreased packed cell volume, reduced erythrocytes, decreased albumin	0.3		

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Crossbred (New Zealand × Chinchilla) male rabbits (49 days old, 0.76 kg)	FB_1 from *F. verticillioides* (MRC 826); FB_2 and FB_3 in feed were negligible and not even detectable in most of the samples	196 days	12	0.13 (control), 5, 7.5 or 10; doubts about chemical analysis by ELISA	Reported to be equal to 0.005, 0.199, 0.292 or 0.373	Diet	Increased % lymphocytes, decreased total serum protein, decreased serum albumin, decreased albumin to globulin ratio, increased alkaline phosphatase, increased relative kidney weight, adverse effects in both kidney and liver	0.199	<0.199 *Owing to many limitations in the studies, the Committee did not consider the NOAELs to be reliable, and these were not used in the evaluation.*	Ewuola & Egbunike (2008); Ewuola (2009)
							Decreased packed cell volume and red blood cell concentration, increased white blood cells, increased serum globulin, increased testes weight	0.292	0.199	
							Increased AST, decreased liver weight	0.373	0.292	

Table 3 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Pigs										
Male weaned piglets (10 kg)	Fumonisins prepared from *F. verticillioides* culture material	4 weeks	5	0, 10, 20 or 40	0, 0.4, 0.8 or 1.6	Diet	Increased serum sphinganine to sphingosine ratios (8 days)	0.4	<0.4	Zomborszky-Kovács et al. (2002a,b)
							Elevated serum AST (28 days)	0.8	0.4	
		8 weeks	5	0, 1, 5 or 10	0, 0.04, 0.2 or 0.4		Increased serum sphinganine to sphingosine ratios (15 days)	0.2	Authors: 0.04 *Committee: NOAEL cannot be determined, conclusions are based on descriptive data that cannot be quantified.*	
							Lung oedema, thickened interlobular septa and peribronchovascular interstitial thickening (8 weeks)	0.2		
							Increased lung weight (8 weeks)	0.4	0.2	
		20 weeks	5	0, 1, 5 or 10	0, 0.04, 0.2 or 0.4		Unclear	Unclear	Unclear	

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Male Yorkshire hybrid pigs (3 weeks old)	*F. verticillioides* (NRRL 34281) culture extract containing FB_1	6 days	9	—	0 or 0.5	Gavage	Significant increase in bacterial colonization in the ileum, caecum and colon, more than with pure FB_1	0.5	<0.5	Oswald et al. (2003)
	Pure FB_1 (>95%)		4 or 5				Significant increase in bacterial colonization in the ileum, caecum and colon, less than with culture material			
Crossbred piglets (7 weeks old, mean 15 kg)	FB_1 and FB_2 from *F. verticillioides* (MRC 826) culture material	28 days	4	0, 10 or 30	0, 0.4 or 1.2	Diet	Differences in feed consumption, weight gain, haematological and biochemical parameters, pulmonary oedema, pathological alterations	1.2	0.4	Dilkin et al. (2003)
Male pigs (Hungarian Large White, 8 weeks old, 12–14 kg)	Fumonisins from *F. verticillioides* (MRC 826) culture material	10 days	6 in control, 10 in test group	0 or mix of 45 (FB_1), 8.6 (FB_2) and 4.6 (FB_3)	0 or mix of 1.8 (FB_1), 0.34 (FB_2) and 0.18 (FB_3)	Diet	Pulmonary oedema, accumulation of fluid in the pleural cavity	1.8 (FB_1) + 0.34 (FB_2) + 0.18 (FB_3)	<1.8 (FB_1) + <0.34 (FB_2) + <0.18 (FB_3)	Fodor et al. (2008)

Table 3 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Male weanling pigs (White, 8–9 weeks old, 7 kg)	FB_1 from *F. verticillioides* (MRC 826) culture material	6 months	6	0, 0.2, 5, 10 or 15	Reported to be equal to 0, 0.2, 6, 11.5 or 17	Diet	Decreased digestibility of ether-extractable nutrients (weaning pigs)	0.2	<0.2	Gbore & Egbunike (2007); Gbore (2009a, 2010)
							Decreased digestibility of ether-extractable nutrients (peri-pubertal pigs), decreased digestibility of dry matter and nitrogen-free extract, increased daily dry matter intake and feed conversion ratios, increased age at puberty (pubertal pigs) Decreased acetylcholinesterase activity in amygdale, medulla oblongata, cerebral cortex and mid-brain, increased activity in hippocampus and hypothalamus. *Note: The Committee concluded that these effects could not be attributed to fumonisin exposure.*	6	0.2 *Owing to limitations in the study, these results were not used in the evaluation.*	

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
							Decreased daily weight gain (weanlings), increased feed conversion ratios (pre-pubertal pigs), decreased daily weight gain, increased weight (pubertal pigs)	11.5	6	
							Decreased final live weight and daily dry matter intake, increased feed conversion ratio (weanlings) Decreased daily weight gain (pre-pubertal pigs) Decreased digestibility of crude protein (peri-pubertal pigs), decreased digestibility of organic matter and crude fibre and increased ash digestibility (pubertal pigs)	17	11.5	
Horses										
Horses of various breeds and sexes (6–24 months old)	FB_1 (>95%)	7–28 days	3–4	—	0, 0.01 or 0.2 mg/kg bw per day	Intravenous	Elevated serum and right ventricular sphinganine and sphingosine concentrations	0.01	<0.01	Smith et al. (2002)

Table 3 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
							Neurological signs consistent with equine leukoencephalomalacia Cardiovascular: decreased heart rate, cardiac output, right ventricular contractility and coccygeal artery pulse pressure Venous blood: significant pH and base excess, increased vascular resistance	0.2	0.01 (NOAEL as determined in study)	
Horses of various breeds and sexes (6 months to 8 years old)	FB_1 (>95%)	28 days	3–4	—	0, 0.01, 0.05, 0.10 and 0.20	Intravenous	Increased serum sphinganine 1-phosphate	0.01	<0.01	Tumbleson et al. (2003); Foreman et al. (2004); Constable et al. (2005)
							Neurological signs characterized by mild proprioceptive abnormalities, including hindlimb ataxia, delayed forelimb placing reactions and decreased tongue tone and movement	0.05	0.01	

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)[a]	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
							Behavioural changes included depression, hyperaesthesia and intermittent dementia; increased levels of protein, albumin and IgG concentrations in the cerebrospinal fluid; lower atlanto-occipital cerebrospinal fluid opening pressure and lower cardiac output; cerebral oedema; increased free sphingoid bases in serum, liver, kidney and other tissues	0.05	0.01	

ALT, alanine aminotransferase; ASP, aspartate aminotransferase; BMD, benchmark dose; ELISA, enzyme-linked immunosorbent assay; IgG, immunoglobulin G; TNFα, tumour necrosis factor alpha

[a] Concentrations in diet were converted to doses using standard JECFA default values. When other conversions were used, this is indicated in the table.

The fumonisin-treated mice showed obvious but mild hepatocellular apoptosis and anisocytosis that were comparable in both uninfected and *T. cruzi*–inoculated mice and comparable dose-dependent increases in the liver sphinganine to sphingosine ratio. Nitric oxide production by peritoneal macrophages from the inoculated mice treated with fumonisin was significantly increased approximately 2 weeks before similar increases in nitric oxide production were seen in the inoculated mice not consuming the fumonisin diet. The inoculated mice consuming the fumonisin diet also had significantly reduced numbers of trypomastigotes in the blood after 26 days, which was paralleled by decreased numbers of pseudocysts in the cardiac muscle. It was concluded that the consumption of the diets containing fumonisins increased host resistance to *T. cruzi* by modulating nitric oxide production by macrophages (Osborne et al., 2002). As effects were seen at the lowest dose, the Committee could not determine a NOAEL for either toxicity or increased host resistance.

Male tumour necrosis factor alpha (TNFα)-null mice and wild-type mice (five animals per group) were fed control diets or diets prepared from *F. verticillioides* (MRC 826) culture material that was determined by HPLC to contain 300–350 mg FB_1 per kilogram diet (equivalent to 45–52 mg/kg bw per day) for 8 days. TNFα-null mice that were fed the fumonisin-containing diet had significantly increased hepatic apoptosis and mitotic figures, approximately 10- and 20-fold greater, respectively, compared with the fumonisin-treated wild-type mice. Liver sphinganine was also significantly higher in the fumonisin-treated TNFα-null mice compared with the fumonisin-treated wild-type mice (Voss et al., 2006b). The results confirm the findings of a study conducted in the same mouse strain but using subcutaneous injection of pure FB_1 (Sharma et al., 2002). The results show that loss of the ability to produce TNFα exacerbates the hepatotoxicity of dietary exposure to fumonisin (Voss et al., 2006b). As effects were seen at the only dose tested, a NOAEL could not be determined by the Committee.

(ii) Rats

Male Sprague-Dawley rats (12 animals per group) were fed test diets prepared using *F. verticillioides* (MRC 826) culture material so that the final concentrations of total fumonisins (FB_1, FB_2 and FB_3 at 1.00:0.45:0.10 by weight) were 1.1, 13.5 or 88.6 mg/kg diet for a total of 10 days (equivalent to 0.11, 1.35 or 8.86 mg/kg bw per day). The fumonisin levels in the test diets, liver, kidney and serum were determined using liquid chromatography (LC) tandem electrospray ionization (ESI) mass spectrometry (MS). Animals were sacrificed on days 1, 3, 5 and 10 (n = 3 per time point per treatment group). After 10 days on the diets, there were no statistically significant differences in body weight or feed consumption among any of the treatment groups. The absolute kidney weights were significantly decreased in the animals consuming diets containing 13.5 and 88.6 mg fumonisins per kilogram. The kidneys of the animals receiving the diet containing 1.1 mg fumonisins per kilogram were unremarkable, and those animals consuming the diets containing total fumonisins at 13.5 mg/kg diet and 88.6 mg/kg diet exhibited histological changes in both liver and kidney consistent with those known to be caused by pure fumonisins. The lesions in the kidney were more severe and appeared at earlier time points than those in the liver. It was found that the amount

of FB_1 detected in kidney was 10 times that detected in the liver, and the same was true for the sphinganine concentrations in kidney and liver. In the kidney, the concentrations of sphinganine and sphinganine 1-phosphate were closely correlated with the pathology scores and the levels of FB_1 in the tissue ($r^2 = 0.78$, $P < 3 \times 10^{-8}$). In the liver, no sphingoid base 1-phosphates were detected, but there was a close correlation between sphinganine concentration, FB_1 concentration and the pathology scores in the liver. Whereas FB_1 was detected in the liver and kidney at all time points, there was no FB_2 or FB_3 detected in the kidney, liver or serum at any time point, suggesting that FB_1 is preferentially accumulated over FB_2 and FB_3 (Riley & Voss, 2006). Based on the lack of significant pathology in the liver and kidney and the lack of any apparent time-dependent increase in sphinganine or sphinganine 1-phosphate in rats consuming the 1.1 mg/kg diet, the Committee concluded that the NOAEL was 0.11 mg/kg bw per day and that the LOAEL was 1.35 mg/kg bw per day (for total fumonisins).

Male Sprague-Dawley rats (10 animals per group, 73–74 g) were fed diets prepared from corn grits and extrusion-cooked corn grits (with and without glucose) from *F. verticillioides* M-2552 with approximately 10 and 50 mg FB_1 per kilogram for either 3 or 8 weeks (Voss et al., 2011). The fumonisin content was determined based on a combination of HPLC with fluorescence detection and liquid chromatography with tandem mass spectrometry (LC-MS/MS) analysis. Mass balance analysis showed that 38–46% of the FB_1 species detected in corn extruded with glucose was the glucose reaction product, *N*-(deoxy-D-33 fructos-1-yl)-FB_1 (Jackson et al., 2011). Diets were prepared by blending (50% weight per weight [w/w]) basal diet (Certified Teklad TD.09.119, 22% total protein) with the corn grits (6–7% total protein) that had been subjected to cooking schemes with and without glucose. The final total protein content of the diets was 14–15%. The purpose of the study was to determine the ability of extrusion cooking alone or with glucose supplementation to reduce the toxicity of fumonisin using the renal toxicity in rats as a bioassay. The FB_1 contents of the low- and high-FB_1 diets were as follows: uncooked and extruded control diets, <0.1 mg/kg; fermented grits extruded with glucose, 0.3 and 2.9 mg/kg diet; fermented grits extruded without glucose, 1.4 and 9 mg/kg diet; fermented grits unextruded, 4.9 and 25 mg/kg diet. Thus, the average levels of FB_1 in the test diets over the 8-week period were <0.1, <0.1, 0.3, 1.4, 2.9, 4.9, 9 and 25 mg/kg diet. Based on body weight and feed consumption data, corresponding average exposures calculated by the authors for the entire 8-week feeding period were <0.01, <0.01, 0.03, 0.10, 0.22, 0.35, 0.70 and 1.80 mg/kg bw per day. After 8 weeks on the test diets, no differences in body weight were found between the uncooked control and other groups. There were some significant differences in feed consumption during the first 3 weeks, but no significant differences were found after week 3. There were no haematology or serum chemistry findings; however, urinalysis revealed signs of kidney dysfunction at 9 and 25 mg/kg diet. Significant decreases in absolute and relative kidney weights compared with the control diets were seen mostly in the higher dose groups. Treatment-related lesions were seen in kidney at both 3 and 8 weeks.

The pathology scores within dose groups were highly variable but still clearly dose and time dependent. At 3 weeks, the dose groups that had pathology scores that were significantly higher than those of the control groups were those

at and above 4.9 mg FB_1 per kilogram diet. Pathology scores increased in a dose-dependent manner at both time points. At 8 weeks, however, because of the animal-to-animal variability in lesion severity, significant increases in pathology scores were demonstrated only in animals consuming the diets containing 4.9 or 25 mg/kg.

Nonetheless, there was a close correlation between the individual pathology scores and their individual FB_1 exposures based on the regression analysis. The degree of ceramide synthase inhibition as measured by the sum of sphinganine plus sphinganine 1-phosphate was closely correlated with FB_1 exposure. Sphinganine plus sphinganine 1-phosphate was also closely correlated with the pathology scores at both weeks 1–3 and weeks 4–8 as based on regression analysis.

The statistical analysis of the exposures versus the sum of renal sphinganine plus sphinganine 1-phosphate showed that the only treatment group that was not significantly different from both control groups was the 0.03 mg/kg bw per day group at both 3 and 8 weeks. The conclusion was that extrusion cooking, especially with glucose supplementation, is effective for reducing fumonisin toxicity and that the results provided no evidence for the formation of toxic "hidden" fumonisins or degradation products during extrusion (Voss et al., 2011). The Committee concluded that, given the variability of the pathology scores and the close correlation between fumonisin exposure, the pathology scores and the levels of total sphingoid bases and sphingoid base 1-phosphates in rat kidney, the NOAEL in this study could be 0.03 mg/kg bw per day. This study was used in the evaluation, and dose–response modelling was performed using the pathology scores and fumonisin exposure data from the study.

Female Wistar rats (13 animals per group) were fed diets prepared from *F. verticillioides* corn grits culture material made to contain 0.2 (control), 10 or 20 mg FB_1 per kilogram for 35 days (calculated by the Committee to be equivalent to 0.02, 0.82 and 1.98 mg/kg bw per day using the average weight and feed intake provided in the manuscript). The concentration of FB_1 in the diets was determined by ELISA and confirmed by HPLC. At the end of 35 days, the final weights and the relative weight gains were significantly less in the animals consuming 10 and 20 mg FB_1 per kilogram compared with the animals consuming the control diet. The daily weight gain and daily feed intake were not significantly different, but the feed conversion ratio was significantly increased in the animals in the high-dose group. Analysis of the chemical composition of faecal samples revealed evidence indicating reduced nutrient digestibility in the rats consuming both the 10 and 20 mg FB_1 per kilogram diets compared with the control diet. It was concluded that FB_1 concentrations at and above 0.82 mg/kg bw per day could adversely affect nutrient utilization and growth performance (Gbore, Yinusa & Salleh, 2010). The Committee determined that the NOAEL was the dose of the control group, 0.02 mg/kg bw per day.

(iii) Rabbits

Crossbred male rabbits (22–24 weeks old, 1.36 ± 0.01 kg, 10 animals per group) were fed diets prepared with maize that had been inoculated with *F. verticillioides* MRC 826 at silking in the field. The diets were prepared with the contaminated maize blended with a defined basal diet to contain 0.35 (control), 12.3 or 24.6 mg fumonisin per kilogram diet (equivalent to 0.011, 0.37 and 0.74

mg/kg bw per day) based on ELISA (Neogen) and were fed to the rabbits for 5 weeks. Presumably the fumonisin concentrations were the concentrations of total fumonisins (FB_1 + FB_2 + FB_3). There were no significant differences in final live weight or weight gain, but there was a significant decrease in dry matter intake and an increase in the feed conversion ratio in the animals consuming the diet containing 24.6 mg fumonisin per kilogram. There were no significant effects on any of the haematological parameters measured. The only statistically significant change in serum chemistry was an increase in serum albumin to globulin ratio in the group fed the 12.3 mg fumonisin per kilogram diet. There were no significant effects on serum enzymes indicative of liver damage (alanine aminotransferase [ALT] and aspartate aminotransferase [AST]). The authors concluded, based on the lack of any dose-dependent changes other than decreased dry matter intake and increased feed conversion ratio at 24.6 mg/kg diet, that the NOAEL for this study was 0.37 mg fumonisin per kilogram body weight per day (Ewuola et al., 2008). The Committee agreed with this conclusion.

Crossbred mature female rabbits (eight animals per group; 1.65–2 kg) were fed diets prepared using *F. verticillioides* (MRC 826) culture material blended with a defined rabbit chow to achieve fumonisin concentrations (based on ELISA, Neogen) of 0, 5 or 10 mg/kg in the test diet (presumably this was total fumonisin, equivalent to 0, 0.15 and 0.3 mg/kg bw per day). Animals were fed the diets for 2 weeks and then mated and fed for an additional 4 weeks. The control animals showed no weight gain over the course of the study, and the animals in the two dose groups actually lost weight. The final weights in both dose groups were significantly less than those of the controls. Dry matter intake was also significantly reduced in the two fumonisin treatment groups, and animals fed 10 mg/kg had dry matter intake that was 50% of the control or 5 mg/kg treatment groups. After 6 weeks on the diets, there were numerous significant differences in haematological parameters in the pregnant does. These included decreased packed cell volume (10 mg/kg), haemoglobin (≥5 mg/kg), erythrocytes (10 mg/kg) and lymphocytes (5 mg/kg) and increased leukocytes (≥5 mg/kg) and neutrophils (5 mg/kg). Serum chemistry changes included decreased total protein (≥5 mg/kg) and albumin (10 mg/kg) and increased ALT and AST (≥5 mg/kg) and alkaline phosphatase (10 mg/kg) activities. Serum electrolytes (phosphate, chloride, bicarbonate) and creatinine also showed significant differences compared with the control group, but the changes were not dose dependent. No information was provided on the reproductive effects or outcome of the pregnancies, even though the authors concluded that the 5 mg/kg diets may negatively influence the proper growth and development of fetuses (Gbore & Akele, 2010). As effects were seen at the lowest dose tested, a NOAEL could not be determined. The Committee noted that there were numerous problems with the statistical analysis, and therefore the study was not used in the evaluation.

Crossbred (New Zealand × Chinchilla, 49 days old, 0.76 kg) male rabbits (12 animals per group) were fed diets prepared using *F. verticillioides* (MRC 826) culture material blended with a defined rabbit chow to achieve fumonisin concentrations (based on ELISA, Neogen) of 0.13, 5, 7.5 and 10 mg/kg in the test diet for 196 days. The daily FB_1 exposure was reported to be equal to 0.005 (control diet), 0.199, 0.292 and 0.373 mg/kg bw. The concentrations of FB_2 and FB_3 were reported to have been negligible and not even detectable in most of the samples.

The purpose of the study was to evaluate the effects of fumonisin on serum chemistry and haematological parameters at 84 days (Ewuola & Egbunike, 2008), organ weights and histopathology at 196 days (Ewuola, 2009) and reproductive effects at 175 and 196 days (Ewuola & Egbunike, 2010a,b). The reproductive effects are presented in section 2.2.5. The effects on haematological parameters assessed at 84 days were minor, with significant decreases in packed cell volume and red blood cell concentration and increased white blood cells at and above 7.5 mg/kg diet. There was a significant increase in the per cent lymphocytes (≥5 mg/kg), which was not dose dependent. There were also significant decreases in total serum protein (≥5 mg/kg), serum albumin (≥5 mg/kg) and albumin to globulin ratio (≥5 mg/kg) and increases in serum globulin (≥7.5 mg/kg), AST (10 mg/kg) and alkaline phosphatase (≥5 mg/kg). At 196 days, there were significant increases in relative kidney weight (≥5 mg/kg) and testes weight (≥7.5 mg/kg) and a significant decrease in liver weight (10 mg/kg). The pathology scores (five animals per group examined) also indicated dose-dependent adverse effects in both kidney and liver in rabbits fed the diets containing 5 mg FB_1 per kilogram and above. In the animals consuming the 10 mg/kg diet, pathology scores (five animals examined) rated as "severe" in liver, kidney, testes, stomach and small intestines were 4/5, 3/5, 0/5, 1/5 and 2/5 (Ewuola & Egbunike, 2010a,b). A NOAEL could not be determined. The Committee considered the lack of confirmation of the fumonisin levels in the diets using a more quantitative method of analysis troubling. There was no confirmation of the results of the ELISA, and the fact that FB_2 and FB_3 were not detected in the diets was considered problematic. Also, there was no statistical analysis of the final body weights, and the Committee observed that the final body weights of the control group and the 10 mg/kg group were similar (1.88 kg versus 1.86 kg), which raises some doubts, given the severity of the pathology reported in liver and kidney in the high-dose group. Owing to these limitations, the Committee decided not to use these studies for the evaluation.

(iv) Pigs

Male weaned piglets (breed unknown, 10 kg, five animals per group) were fed diets containing fumonisins prepared from *F. verticillioides* culture material for up to 20 weeks. The diets were prepared to contain FB_1 concentrations ranging from 0 to 40 mg/kg in three separate experiments of differing duration. In the first trial, animals were dosed for 4 weeks at 0, 0.4, 0.8 or 1.6 mg/kg bw per day; in the second trial, animals were dosed for 8 weeks at 0, 0.04, 0.2 or 0.4 mg/kg bw per day; and in the third trial, animals were dosed for 20 weeks at 0, 0.04, 0.2 or 0.4 mg/kg bw per day.

In the 28-day study, the pigs fed the diets containing 20 mg FB_1 per kilogram and higher had significantly elevated serum AST levels at 28 days and dose-dependent increases in serum sphinganine to sphingosine ratios that were significant in groups fed diets containing 10 mg FB_1 per kilogram diet and higher. After 8 weeks, there was a trend towards increased lung weight in the animals fed the 10 mg/kg diet. Oedema and morphological changes apparent from computer tomography scans, including thickened interlobular septa and peribronchovascular interstitial thickening, suggestive of adverse effects in the lung, were described in animals consuming the diets that contained 5 mg/kg and above. The authors

concluded that the data indicated a NOAEL of 1 mg/kg diet, equivalent to 0.04 mg/kg bw per day (Zomborszky-Kovács et al., 2002a,b). The Committee concluded that many of the conclusions were based on descriptive information that cannot be quantified. The relative incidence of macroscopic lesions had no control data and no statistical analysis, and the levels of FB_2 and FB_3 were not given. These studies were therefore not used in the evaluation.

Male Yorkshire hybrid pigs (3 weeks old) were gavaged for 6 days with either *F. verticillioides* (NRRL 34281) culture extract containing FB_1 or pure FB_1 (>95% purity) at 0.5 mg/kg bw per day. The level of FB_1 in the culture extracts was determined by quantitative thin-layer chromatography (TLC). The levels of FB_2 and FB_3 in the gavaged culture extracts were not reported. On the last day of gavage with fumonisin, the pigs were inoculated with a pathogenic strain of *Escherichia coli*, and 24 hours later, the animals were euthanized. There were no significant treatment-related effects on body weight gain, clinical signs, performance, gross changes at necropsy or microscopic lesions in liver or other tissues compatible with FB_1 toxicity. There were no treatment-related changes noted in the plasma biochemical analysis. Examination of the intestines 24 hours after inoculation with *E. coli* revealed that treatment with either the culture extract containing FB_1 or pure FB_1 resulted in a significant increase in bacterial colonization in the ileum, caecum and colon. The extent of the colonization and translocation to extraintestinal organs was greater in the animals receiving the FB_1 in the culture extracts compared with the pure FB_1. The authors concluded that FB_1 is a predisposing factor to infectious disease (Oswald et al., 2003). The NOAEL for toxicity under the experimental conditions described is 0.5 mg FB_1 per kilogram body weight per day. A NOAEL for increased susceptibility to bacterial intestinal colonization could not be determined.

Crossbred piglets (two of each sex per group, 7 weeks old, mean 15 kg) were fed diets prepared from *F. verticillioides* (MRC 826) culture material containing a total of 10 mg fumonisins (FB_1 and FB_2) per kilogram diet or 30 mg fumonisins per kilogram diet for 28 days. This study was conducted to determine the possible interactive effects of diets co-contaminated with fumonisins and AFB_1; only the effects of consuming the fumonisins-only diets are summarized in this review. The concentrations of FB_1 (26 and 8 mg/kg diet, respectively) and FB_2 (8 and 3 mg/kg diet, respectively) in the high- and low-dose diets, respectively, were determined by HPLC with fluorescence detection. Statistically significant differences in feed consumption, weight gain, and haematological and biochemical parameters were seen predominantly in the group fed the 30 mg/kg diet compared with the control-treated animals, and one animal consuming the 30 mg/kg diet died of pulmonary oedema. Pathological alterations were observed only in animals fed the 30 mg/kg diet. It was concluded that 10 mg fumonisins per kilogram feed is safe for swine (Dilkin et al., 2003). The NOAEL was 10 mg FB_1 and FB_2 per kilogram diet, equivalent to 0.4 mg/kg bw per day.

Pigs (male Hungarian Large White, 12–14 kg) were fed a diet prepared from *F. verticillioides* (MRC 826) culture material for 10 days. Diets were analysed by HPLC with fluorescence detection and contained 45 mg of FB_1, 8.6 mg of FB_2 and 4.6 mg of FB_3 per kilogram. All piglets developed mild signs of pulmonary oedema, including the accumulation of fluid in the pleural cavity, which persisted in several

animals 10 days after removal from the contaminated diets. A NOAEL could not be determined (Fodor et al., 2008).

Male weanling pigs (White, 8–9 weeks of age, 7 kg) were fed four diets (six per group) containing 0.2, 5, 10 or 15 mg FB_1 per kilogram prepared from *F. verticillioides* (MRC 826) culture material for 6 months. The level of fumonisin in the diet was determined using ELISA (Neogen). As reported in Gbore (2009a), the average daily dietary exposure to FB_1 was equal to 0.2, 6, 11.5 and 17 mg/kg bw per day. The purpose of the study was to evaluate the effects of fumonisin on nutrient utilization (Gbore & Egbunike, 2007), growth performance (Gbore, 2009a), brain neurotransmitter levels (Gbore, 2010) and semen quality and sperm production (Gbore & Egbunike, 2008; Gbore, 2009b). The effects on semen quality and sperm production are presented in section 2.2.5. The effects on brain neurotransmitter levels are described in section 2.2.6(b). In terms of nutrient digestibility, weanling pigs fed the 5, 10 and 15 mg/kg diets showed a significant decrease in digestibility (<10%) of ether-extractable nutrients compared with the animals fed the diet containing 0.2 mg/kg. In the peri-pubertal pigs, digestibility of crude protein and ether-extractable nutrients was significantly decreased at 15 and 5 mg/kg diet, respectively. In the pubertal boars, significant decreases in digestibility of dry matter (≥5 mg/kg), organic matter (15 mg/kg), crude fibre (15 mg/kg) and nitrogen-free extract (5 and 15 mg/kg) and increased ash digestibility (15 mg/kg) were reported. In weanling pigs, significant decreases in the final live weight (15 mg/kg), daily dry matter intake (15 mg/kg) and daily weight gain (≥10 mg/kg) and a significant increase in the feed conversion ratio (15 mg/kg) were reported. In pre-pubertal pigs, daily weight gains were significantly decreased at 15 mg/kg, and feed conversion ratios were increased at and above 10 mg/kg. In the pubertal boars, daily dry matter intake and feed conversion ratios were significantly increased (≥5 mg/kg), daily weight gain was decreased (≥10 mg/kg) and the weight (≥10 mg/kg) and age at puberty (≥5 mg/kg) were increased. It was concluded by the authors that FB_1 levels of 5 mg/kg diet (reported to be equal to 6 mg/kg bw per day) and above could adversely affect the growth and performance of pigs (Gbore & Egbunike, 2007; Gbore, 2009a, 2010). The Committee noted that the levels of fumonisins in the diet were determined by ELISA without confirmation using a more quantitative method of analysis. Thus, the dosages were uncertain, precluding the use of this study for the evaluation.

(v) Horses

Healthy horses of various breeds and sexes ($n = 11$) ranging in age from 6 to 24 months (252–367 kg) were dosed daily using intravenous administration of FB_1 (>95% pure) at 0 (four animals), 0.01 (three animals) or 0.2 (four animals) mg/kg bw per day for 7–28 days. The purpose of the study was to assess cardiovascular changes in horses that developed signs of neurological disease consistent with equine leukoencephalomalacia (ELEM). Horses dosed with 0.2 mg/kg bw per day developed neurological signs consistent with ELEM 7–9 days after dosing began and were euthanized along with a paired control horse. Horses dosed with 0.01 mg/kg bw per day never developed neurological signs and were euthanized on day 28. Significant cardiovascular differences between the control group and the 0.2 mg/kg bw per day group included decreased heart rate, cardiac output, right ventricular contractility and coccygeal artery pulse pressure. Venous blood showed significant pH and base excess and increased vascular resistance compared

with controls. The only fumonisin treatment–related effect in the 0.01 mg/kg bw per day group was significant elevation in serum and right ventricular sphinganine and sphingosine concentrations, which was also seen in the high-dose group but of much greater magnitude. It was concluded by the authors that cardiovascular changes may contribute to development of ELEM (Smith et al., 2002).

A mix of breeds and sexes (n = 17, one male, geldings and females) ranging in age from 6 months to 8 years (252–524 kg) were dosed daily using intravenous administration of FB_1 (>95% pure) at 0 (control, four animals), 0.01 (three animals), 0.05 (three animals), 0.10 (three animals) and 0.20 (four animals) mg/kg bw per day for up to 28 days. All horses were in good health at the start of the study. All horses receiving 0.05 mg/kg bw or higher (10 animals) developed neurological signs characterized by mild proprioceptive abnormalities, including hindlimb ataxia, delayed forelimb placing reactions and decreased tongue tone and movement. Behavioural changes, including depression, hyperaesthesia and intermittent dementia, were seen at doses of 0.05 mg/kg bw per day and higher. Neurological abnormalities were greater in the horses receiving the higher doses. Horses exhibiting neurological signs had higher levels of protein, albumin and IgG concentrations in the cerebrospinal fluid. Horses exhibiting neurological disease had lower atlanto-occipital cerebrospinal fluid opening pressure, which was significantly correlated with lower cardiac output. Upon histological and postmortem examination, horses with neurological disease had cerebral oedema. The oedema, cardiovascular changes and high levels of cerebrospinal fluid albumin and albumin quotient in horses with neurological disorders were supportive of the conclusion that the cause of the brain lesion was vasogenic cerebral oedema and brainstem or cerebellar herniation into the vertebral canal. Free sphingoid bases in serum, liver, kidney and other tissues and serum sphinganine 1-phosphate levels were significantly elevated, with serum sphinganine 1-phosphate being elevated at all dose levels (Tumbleson et al., 2003; Foreman et al., 2004; Constable et al., 2005). The Committee determined that the NOAEL (intravenous) after 28 days for clinically defined neurological signs was 0.01 mg/kg bw per day.

(vi) Other species

Mule ducks (7 days old) were gavaged for 77 days with *F. verticillioides* (NRRL 34281) culture extract that was partially purified and contained FB_1 at doses of 0, 2, 8, 32 and 128 mg/kg feed. The purity of the FB_1 was 54%, and the extract also contained 8% FB_2 and 9% FB_3. Body weight gain was significantly affected between 7 and 63 days in the ducks dosed with 128 mg FB_1 per kilogram feed and between 28 and 63 days in the animals receiving 32 mg/kg feed. The lowest dose to cause a significant effect on relative organ weight was 32 mg/kg feed, and effects were seen in the liver and spleen. Alkaline phosphatase activity was significantly elevated at and above 32 mg/kg feed. The sphinganine to sphingosine ratio was significantly elevated in serum, liver and kidney at and above 8 mg/kg feed (Tran et al., 2005). The NOAEL was 8 mg/kg diet. The equivalent dose in milligrams per kilogram body weight per day could not be calculated, but, assuming that the duck is equivalent to a chick, it would be 1 mg/kg bw per day.

Male turkeys (7 days old, BUT 9 strain) were fed diets prepared from fumonisin-contaminated corn containing FB_1 and FB_2 at 0, 5, 10 and 20 mg/kg diet

for 63 days. No significant adverse effects on body weight gain, serum chemistry, macroscopic lesions or histological lesions in liver or kidney were found (Tardieu et al., 2007). The NOAEL was greater than 20 mg/kg diet. The equivalent dose in milligrams per kilogram body weight per day could not be calculated, but, assuming that the duck is equivalent to a chick, it would be 2.5 mg FB_1 + FB_2 per kilogram body weight per day.

2.2.3 Long-term studies of toxicity and carcinogenicity

Since the 2001 evaluation (Annex 1, reference *153*), there have been no long-term carcinogenesis bioassays (chronic exposure for most of the lifespan of an animal) to determine the potential carcinogenic hazard and dose–response relationships of fumonisins.

A working group convened by the International Agency for Research on Cancer (IARC, 2002) reached the following conclusions about the carcinogenicity of FB_1:

- There is inadequate evidence in humans for the carcinogenicity of fumonisins.
- There is sufficient evidence in experimental animals for the carcinogenicity of FB_1.
- Overall evaluation: FB_1 is possibly carcinogenic to humans (Group 2B).

2.2.4 Genotoxicity

Since the previous evaluation by the Committee (Annex 1, reference *153*), a number of in vitro and in vivo studies have been published concerning the genotoxicity of FB_1. An overview of these studies can be found in Tables 4 and 5.

A study by DeLorenzi et al. (2005) indicated that FB_1 increased the mitotic index and micronucleus formation in bovine lymphocytes. However, the study is poorly described, and it is questionable whether the method used is suitable.

A study by Rumora et al. (2002) suggested an increased micronucleus formation in rabbit kidney RK13 cells. However, the cell cultures showed a viability much less than 50%. Studies at such low levels of cell viability are prone to detect false-positive effects. Therefore, the study cannot be deemed sound.

The new studies show a number of positive effects. An in vitro study in C6 glioma cells and MEF cells indicated deoxyribonucleic acid (DNA) adduct formation (8-hydroxy-2′-deoxyguanosine [8-OH-dG]) following lipid peroxidation induced by FB_1 (Mobio et al., 2003). Similar studies showing positive effects following lipid peroxidation have been reported in the forty-seventh JECFA evaluation (Annex 1, reference *125*). It is considered that the lipid peroxidation indicates a non-genotoxic (or indirect genotoxic) mechanism of action of fumonisin. The study of Ehrlich et al. (2002) with the HepG2 cells may indicate that FB_1 has a clastogenic effect.

In an in vivo study measuring DNA breaks with the comet assay, Domijan et al. (2007a) showed that FB_1 caused DNA lesions in the kidney. The authors pointed to a non-genotoxic effect, suggesting an important role of sphingolipids in the DNA damage caused by FB_1. In a second in vivo study by Domijan et al. (2008), an increase in DNA damage as demonstrated by a comet assay was observed in

Table 4. Results of assays for the genotoxicity of fumonisin B_1

End-point	Test system	Concentration	Results	Reference
In vitro				
Reverse mutation	*Salmonella typhimurium* TA98, TA100, TA102, TA1535 and TA1537	FB_1: 25–200 µg/ml (± HepG2-derived enzyme [S9] mix)	Negative	Ehrlich et al. (2002)
8-OH-dG and DNA fragmentation	C6 glioma cells (with normal p53 status) p53-null mouse embryonic MEF cells	C6 cells: 3–36 µmol/l FB_1 MEF cells: 3–18 µmol/l FB_1	Positive[a]	Mobio et al. (2003)
Micronucleus	Human-derived hepatoma (HepG2) cells	25–200 µg/ml	Positive[b]	Ehrlich et al. (2002)
In vivo				
DNA damage in the kidney (comet assay)	Male Wistar rats	Intraperitoneal FB_1 doses (500 µg/kg bw per day for 7 days)	Positive[c]	Domijan et al. (2007a)
DNA damage in the liver (comet assay)	Male Wistar rats	Single oral (gavage) FB_1 doses (5, 50 and 500 µg/kg bw)	Positive[d]	Domijan et al. (2008)

8-OH-dG, 8-hydroxy-2′-deoxyguanosine; DNA, deoxyribonucleic acid; S9, 9000 × *g* rat liver supernatant

[a] Increased 8-OH-dG formation and DNA fragmentation were found, which are likely to be the result of increased formation of reactive oxygen species. The results suggest a possible loss of protective mechanisms (such as p53-dependent apoptosis and cell cycle arrest) in FB_1-damaged MEF cells and confirm that cells lacking mechanisms governed by the *p53* gene would be more susceptible to neoplastic cascade or mutation following DNA lesions induced indirectly by this mycotoxin.

[b] The results may indicate that FB_1 is clastogenic in human-derived cells, although a non-genotoxic effect (aneuploidy) cannot be excluded.

[c] Increased DNA damage was observed in the kidney, as demonstrated by the comet assay. The ratio of sphinganine to sphingosine was also significantly increased. The DNA effects preceded effects on catalase activity and the concentration of protein carbonyls and malondialdehyde. The study authors concluded that disturbed sphingolipid metabolism induced by FB_1 may play a role in the observed DNA damage.

[d] Increased DNA damage was observed in the liver, as demonstrated by the comet assay. Apoptosis in rat liver appeared 24 hours after a single oral FB_1 dose of 5 µg/kg bw. Its appearance was time and dose dependent. As apoptosis appeared before DNA damage, the study authors concluded that FB_1-induced apoptosis is not primarily caused by DNA damage. Mitotic figures seen at low doses of FB_1 support the conclusion that regenerative processes are involved in FB_1 carcinogenesis, as they increase DNA replication. This conclusion supports the mechanism of carcinogenesis summarized in the previous evaluation by the Committee (Annex 1, reference *153*) and the IARC (2002) evaluation.

Table 5. Results of additional assays with end-points related to the genotoxicity of fumonisin B_1

End-point	Test object	Concentration	Results	Reference
In vitro				
Transformation (initiation and promotion)	Bhas 42 cells	FB_1 (0.5–20 µg/ml), for 3 days (initiation assay) FB_1 (0.5–20 µg/ml), for 3 days, followed by 11 days of incubation with FB_1 at 0.5–10 µg/ml	Initiation: Negative Promotion: Positive	Sakai et al. (2007)
Cytotoxicity/apoptosis/cell cycle distribution	K562 human erythroleukaemia cell line	Not reported; effects are expressed as ID_{50}	Positive[a]	Minervini, Fornelli & Flynn (2004)
In vivo				
Induction of $GSTP^+$ foci	Male F344 rats	Initiation phase: control diet or diet containing FB_1 at 250 mg/kg for 3 weeks Promotion phase: control diet or diet containing phenobarbital at 500 mg/kg for up to 30 weeks	Positive[b]	Gelderblom et al. (2008)

$GSTP^+$, placental glutathione *S*-transferase positive; ID_{50}, median inhibitory dose

[a] The study authors concluded that DNA damage and apoptosis rather than plasma membrane damage and necrosis may be responsible for the observed cytotoxicity.

[b] Three weeks of dietary treatment with FB_1 (250 mg/kg feed) induced $GSTP^+$ foci in the liver. The cancer initiation by FB_1 was associated with hepatotoxicity. Subsequent treatment with a promoting regimen (phenobarbital, 0.05% in diet for up to 30 weeks) stimulated the outgrowth of the $GSTP^+$ foci. In the absence of a promoting regimen (phenobarbital), reversion of the $GSTP^+$ foci was observed.

liver. As a single oral dose of FB_1 induces lipid peroxidation and apoptosis in the liver, the genotoxic finding is considered to be the result of this FB_1-induced lipid peroxidation and apoptosis.

Three new tests measuring related end-points are available. The transformation test by Sakai et al. (2007) indicated that FB_1 did not induce an increase in the number of transformed foci in the initiation test, whereas it was positive in the promotion assay. The transformation test is considered to be an in vitro test indicative of the carcinogenic potency of a substance, recognizing both genotoxic and non-genotoxic carcinogens.

A study in the K562 human erythroleukaemia cell line suggests that human blood cells are sensitive to FB_1 exposure and that DNA damage and apoptosis rather than plasma membrane damage and necrosis may be responsible for the observed cytotoxicity (Minervini, Formelli & Flynn, 2004).

Gelderblom et al. (2008) showed that FB_1 induced $GSTP^+$ foci in the liver, associated with hepatotoxicity. Subsequent treatment with phenobarbital stimulated outgrowth of these foci. In the absence of a promoter, reversion of the $GSTP^+$ foci was observed. The authors concluded that, although it has been suggested that fumonisins may have cancer-promoting potential via epigenetic mechanisms involving membrane lipid alterations, indirect genotoxic properties of FB_1, presumably via oxidative damage, should also be considered.

During the previous meeting of the Committee at which fumonisins were evaluated, it was concluded that in a small number of studies in vitro and a single study in vivo, neither FB_1 nor any other fumonisin was shown unequivocally to be genotoxic. Similarly, no direct adducts of fumonisin with DNA had been found, although the increase in 8-OH-dG adducts observed in newly available studies is indicative of oxidative damage. The newly available data generally support the idea that FB_1 is probably not genotoxic (but probably indirectly genotoxic).

2.2.5 Reproductive and developmental toxicity

At the time of the writing of the 2001 evaluation, there was little concern about fumonisin as a reproductive or developmental toxin. To a large part this was due to the fact that embryotoxicity in vivo using animal models was, with few exceptions, secondary to maternal toxicity, and there was no evidence that fumonisin crossed the placenta (Annex 1, reference *153*; IPCS, 2000). Nonetheless, the Committee recommended additional research on the ability of fumonisin to alter placental folate transport to the fetus in vivo and to investigate the relationship between fumonisin exposure and NTDs. The concern about fumonisin as a developmental toxin stemmed largely from the fact that FB_1 was shown in vitro in cultured cells to disrupt folate processing via the high-affinity folate transporter. The high-affinity folate transporter is found in sphingolipid-enriched lipid rafts located in cell membranes. Thus, fumonisin inhibition of ceramide biosynthesis was plausibly linked to folate sufficiency, and folate-deficient diets are associated with increased risk for NTDs (reviewed in Marasas et al., 2004; Gelineau-Van Waes et al., 2009; Voss, Riley & Gelineau-Van Waes, 2011). Studies on the reproductive and developmental toxicity of fumonisins are summarized in Table 6.

Table 6. Overview of studies on reproductive and developmental toxicity of fumonisins

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Studies using pure/purified FB_1										
Pregnant inbred LM/Bc mice	FB_1 (90–95%)	Dosing at embryonic days 7.5 and 8.5, fetus collection at embryonic day 17.5	10	—	0, 5, 10, 15 and 20	Intraperitoneal	NTDs (exencephaly)	5	<5	Gelineau-Van Waes et al. (2005)
Pregnant CD-1 mice	FB_1 (>95%)	Dosing at embryonic days 7 and 8, fetus collection at embryonic day 17.5	10	—	1st trial: 15, 30 or 45 2nd trial: 10, 23, 45 or 100	Intraperitoneal	NTDs (exencephaly) (dose-dependent manner) Elevation in sphinganine in placenta and embryos, inhibition of folate uptake in embryos and placenta	10 30	<10 15	Voss, Riley & Gelineau-Van Waes (2007, 2011)
Studies with HFB_1										
Pregnant LM/Bc mice	HFB_1 prepared from pure FB_1 (>95%)	Dosing at embryonic days 7 and 8, fetus collection on embryonic days 9 and 16	10	—	0, 2.5, 5, 10 or 20 Positive control group: 10 mg FB_1/kg bw	Intraperitoneal	HFB_1: no effects Positive control (FB_1): Decreased weight gain, liver lesions, exencephaly, increased late fetal deaths, decreased average fetus weight, increased sphingolipid metabolites in liver, reduced total complex sphingolipids	—	20, highest dose tested (HFB_1)	Voss et al. (2009)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Pregnant rats from mating of male (326–350 g) and female (201–225 g) caesarean-derived rats	HFB_1 (97.3%)	Embryonic days 3–16	30–31	—	0, 15, 30, 60 or 120	Gavage	Maternal: reduced body weight gain	30 (maternal toxicity)	15 (maternal toxicity); 120 (fetal toxicity)	Collins et al. (2006)
							Maternal: reduced feed consumption	60	30	
							No significant effects on gravid uterine weight, reproductive indices, early or late deaths No fetal, skeletal or soft tissue changes indicative of any teratogenic or developmental effect No changes in the sphinganine to sphingosine ratio in dams or fetal tissue, and no histological effects in dams	—	120, highest dose tested	

Table 6 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Studies with culture material										
Female LM/Bc and CD-1 mice (sexually mature and the same age)	FB_1 from *F. verticillioides* culture material (MRC 826); FB_2 and FB_3 were present in the diets at concentrations equal to about 0.3 of the FB_1 concentration	Dosing at 5 weeks before mating and then after mating until embryonic day 16 at the conclusion of organogenesis	5	0, 50 or 150	Equal to 0, 8 or 25 (LM/Bc strain); 0, 13 or 39 (CD-1 strain)	Diet	LM/Bc strain: NTDs (exencephaly) (in one mouse), mild maternal hepatotoxicity, elevated sphinganine and sphinganine 1-phosphate CD-1 strain: elevated fetal death, mild maternal hepatotoxicity	25 (LM/Bc strain) — (CD-1 strain)	8 (LM/Bc strain) 39, the highest dose tested (CD-1 strain) NOAEL based on NTDs and elevation of sphinganine and sphinganine 1-phosphate	Voss, Gelineau-Van Waes & Riley (2006); Voss, Riley & Gelineau-Van Waes (2011)
				Follow-up: 0, 150 or 300 (only LM/Bc strain)	Follow-up, same protocol: equivalent to 0, 22.5 or 45		Follow-up: no NTDs	—	300, the highest dose tested *In the opinion of the Committee, the results of the second study make the setting of a NOAEL for NTD induction based on the results of the first study uncertain*	

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Pregnant Sprague-Dawley rats (200–210 g, 3 months old)	Fumonisins prepared from *F. verticillioides* culture material (individual levels of FB_1, FB_2 and FB_3 were not reported)	Treatment during embryonic days 6–15, effects measured at embryonic day 20	10	0 or 150 with or without either garlic extract or cabbage extract	Equivalent to 0 or 7.5	Diet	Maternal: reduced body weight, feed intake and litter weight Fetal: effect on number of dead fetuses, live fetuses, fetal body weight and skeletal malformations as a result of maternal toxicity	7.5	<7.5 *The Committee considers the conclusions incorrect, as authors waited for measurements until 5 days after dosing*	Abdel-Wahhab et al. (2004)
Crossbred (New Zealand × Chinchilla) male rabbits (49 days old, 0.76 kg)	FB_1 from *F. verticillioides* (MRC 826); FB_2 and FB_3 in feed are negligible and not even detectable in most of the samples	175 or 196 days	12	0.13 (control), 5, 7.5 or 10 *Doubts about chemical analysis by ELISA*	Equal to 0.005, 0.199, 0.292 or 0.373	Diet	Day 175: delayed onset of puberty and numerous effects indicative of impaired reproductive function, including decreased testicular weight, reduced sperm motility, increased sperm mortality and morphological abnormalities, and increased embryo mortality in does mated to bucks	0.292	0.199 *Doubts about chemical analysis by ELISA*	Ewuola (2009); Ewuola & Egbunike (2010a,b)

Table 6 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
							Day 196: dose-dependent decrease in gonadal and epididymal sperm reserves	0.292	0.199	
							Day 196: decreased sperm productivity seen at doses ≥5 mg fumonisin/kg diet (Ewuola & Egbunike, 2010b)	0.199	<0.199	
							Day 196: elevated testes weights	0.292	0.199	
Male weanling pigs (White, 8–9 weeks old, 7 kg)	FB_1 prepared from *F. verticillioides* (MRC 826) culture material	6 months	6	0.2 (control), 5, 10 or 15	Equal to 0.2 (control), 6, 11.5 or 17	Diet	Increased daily dry matter intake, increased feed conversion ratios, increased age at puberty, effects on semen quality	6	0.2 (NOAEL as determined in study)	Gbore & Egbunike (2008); Gbore (2009b)
							Decreased daily weight gain, increased weight at puberty, effects on sperm production	11.5	6	

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
In vitro study										
ICR mouse embryos (3–5 somite stage)	FB_1 (90–95%), with or without folinic acid	26 h treatment (all doses) or 2 h (50 µmol/l)	10–36 (26 h), 18–25 (2 h)	—	0, 1, 2, 3, 5, 7, 15, 25, 50 or 100 µmol/l	—	Increase in malformations (NTDs; exencephaly), growth retardation with minimal effects on somite development (26 h)	2 µmol/l	1 µmol/l	Sadler et al. (2002)
							Induction of NTDs, facial defects and growth retardation (2 h)	50 µmol/l	25 µmol/l	

NTDs, neural tube defects

(a) Studies using purified fumonisin B_1

(i) Mice

Pregnant inbred LM/Bc mice were dosed (10 animals per dose) intraperitoneally with FB_1 (90–95% pure) on embryonic days 7.5 and 8.5 at 0, 5, 10, 15 or 20 mg/kg bw per day, and fetuses were collected on embryonic day 17.5. Fetuses with NTDs (exencephaly) were seen at all dose levels (5% at 5 mg/kg bw per day to 79% at 20 mg/kg bw per day). NTDs (20% incidence) were also induced by FB_1 gavaged on embryonic days 7.5 and 8.5 at 20 mg/kg bw per day. Significant elevation in sphinganine was detected in placenta and embryos from dams dosed with 20 mg/kg bw per day on embryonic days 7.5 and 8.5 and harvested on embryonic day 10.5. The facts that sphinganine was elevated in embryos and that in a preliminary experiment ^{14}C-labelled FB_1 injected intraperitoneally was detected in embryos on embryonic day 10.5 were consistent with the conclusion that FB_1 crossed the placenta and inhibited ceramide synthase. Significant elevation in sphinganine was also detected in maternal liver and kidney, but the pathology in liver and kidney was not assessed. Studies using [^{3}H]folate showed that FB_1 treatment (20 mg/kg bw per day intraperitoneally) inhibited folate uptake in embryos and placenta and that supplementation with folic acid partially reversed the induction of NTDs (by 50–79%). Supplementation with ganglioside GM1 (embryonic days 6.5–9.5) restored the ability of placenta and embryo to accumulate [^{3}H]folate and was even more effective than folate alone at reducing NTD incidence (79% without GM1 to 5% with GM1). Based on immunohistochemistry, GM1 and the folate binding protein co-localized in the yolk sac membrane, suggesting that GM1 restored lipid raft function and confirming that the disruption of sphingolipid metabolism is the cause of the NTDs (Gelineau-Van Waes et al., 2005). No oral NOAEL could be derived from this study. In the opinion of the Committee, the oral dose that induced NTDs (20 mg/kg bw per day) is within the range of the dietary levels for hepatotoxicity in female mice, indicating that maternal toxicity was likely to be involved (Annex 1, reference *153*, Table 4). In a teratology study (Reddy et al., 1996, reviewed in Annex 1, reference *153*) in which pregnant CD-1 mice were given oral doses of FB_1 daily during embryonic days 7–15, maternal toxicity (hepatic lesions and elevated sphinganine to sphingosine ratio) was found at and above 25 mg/kg bw, and mortality was noted at the two highest levels of 50 and 100 mg/kg bw. Dose-dependent effects, which did not include exencephaly, were found in fetuses only at doses that were maternally toxic (≥25 mg/kg bw). Thus, it is likely that transient maternal toxicity accompanied the induction of NTDs in this study as well.

Using the same intraperitoneal dosing regimen as used by Gelineau-Van Waes et al. (2005), pregnant CD-1 mice were dosed with FB_1 (>95% pure) at 0, 15, 30 or 45 mg/kg bw per day on embryonic days 7 and 8 (equivalent to embryonic days 7.5 and 8.5 in Gelineau-Van Waes et al., 2005) and in a second trial at 0, 10, 23, 45 or 100 mg/kg bw per day. As with the LM/Bc mice (Gelineau-Van Waes et al., 2005), NTDs (exencephaly) were induced in a dose-dependent manner. The CD-1 strain was, however, more resistant to NTD induction: the percentage of NTD-affected litters in LM/Bc dams given 20 mg/kg bw per day and in CD-1 dams given

23 mg/kg bw per day was 100% (10/10) and 17% (2/12), respectively (Voss, Riley & Gelineau-Van Waes, 2007, 2011). A NOAEL could not be derived from this study by the Committee.

(b) Studies using hydrolysed fumonisin B_1

(i) Mice

Pregnant LM/Bc mice were dosed with HFB_1 prepared from pure FB_1 (>95%). The purity of the HFB_1 was determined by a combination of HPLC with fluorescence detection and LC ESI tandem MS (LC-ESI-MS/MS) and comparison with a pure standard. Dams (10 animals per group) were intraperitoneally dosed on embryonic days 7 and 8 with 2.5, 5, 10 or 20 mg HFB_1 per kilogram body weight per day. A positive control group received 10 mg FB_1 per kilogram body weight per day on embryonic days 7 and 8. One half of the females were euthanized on embryonic day 9, and the remainder on embryonic day 16. Weight gain of the FB_1-treated group (positive control) was significantly decreased on embryonic day 16. The positive control dams were the only ones to show significant liver lesions on embryonic day 9 (judged moderately severe), which were consistent with the known effects of FB_1. Hepatotoxic effects were readily reversible; by embryonic day 16, the hepatic lesions in the positive control group were judged minimal. No liver lesions attributable to HFB_1 treatment were found on embryonic day 9 or 16. No fetuses with NTDs were found in the control or any HFB_1-treated group. In contrast, all litters (100%) from the positive control group had at least one affected fetus (all exencephaly). In addition to the NTDs, the positive control group showed significantly increased late fetal deaths and decreased average fetus weight compared with all the HFB_1 treatment groups. No significant differences were seen between the vehicle control and the HFB_1-treated groups at embryonic day 16 for early resorptions per litter, late deaths per litter, live deaths per litter, or live fetal and placental weights. Compared with the positive control, the increases in sphingolipid metabolites indicative of ceramide synthase inhibition in the livers of the dams on embryonic day 9 were minor in the HFB_1 treatment groups. The effect was dose related at and above 5 mg/kg bw per day, but significant ($P < 0.05$) only at the high dose of 20 mg/kg bw per day. For example, the levels of sphinganine, 1-deoxysphinganine and sphinganine 1-phosphate in the 20 mg HFB_1 per kilogram body weight per day treatment group on embryonic day 9 were 29-, 8- and 42-fold less than in the livers of the dams in the positive control group. Likewise, the levels of total complex sphingolipids were not decreased in the livers of HFB_1-treated dams, but were reduced 80–90% in the livers of the positive controls. The authors concluded that the results provide evidence that hydrolysed fumonisins are less toxic in vivo than their parent fumonisins and that they are not a significant risk factor for NTDs (Voss et al., 2009). The NOAEL for HFB_1-induced NTDs and liver toxicity in mice is greater than 20 mg/kg bw per day when dosed for 2 days intraperitoneally. In the Committee's opinion, the data provide additional support for the findings of Howard et al. (2002), summarized in section 2.2.2, and Collins et al. (2006), summarized below.

(ii) Rats

Male (326–350 g) and female (201–225 g) caesarean-derived viral antibody–free rats (breed unknown, from Charles River Laboratories, Inc.) were mated, and pregnant rats were assigned to control or one of four treatment groups (30–31 animals per group). Animals were dosed by gavage on embryonic days 3–16 with 0, 15, 30, 60 or 120 mg HFB_1 per kilogram body weight per day. The HFB_1 was 97.3% pure. Significantly reduced feed consumption was evident in the 60 and 120 mg/kg bw per day dosed animals killed on embryonic days 17 and 20, and body weight gain was significantly reduced in the 30, 60 and 120 mg/kg bw per day groups on embryonic day 17 and the 60 and 120 mg/kg bw per day groups on embryonic day 20. There were no significant effects on gravid uterine weight, reproductive indices, early deaths or late deaths. There were no fetal, skeletal or soft tissue changes indicative of any teratogenic or developmental effect. There were also no changes in the sphinganine to sphingosine ratio in dams or fetal tissue and no histological effects in dams (Collins et al., 2006). The Committee derived a NOAEL for fetal toxicity of 120 mg HFB_1 per kilogram body weight per day and for maternal toxicity of 15 mg/kg bw per day based on the significant decrease in weight gain seen at 30 mg/kg bw per day in dams at embryonic day 17.

(c) Studies using diets containing fumonisin B_1 prepared from F. verticillioides *culture material*

(i) Mice

LM/Bc and CD-1 mice (five animals per dose group, sexually mature and the same age) were fed diets containing *F. verticillioides* culture material (MRC 826) to provide (calculated concentration based on HPLC analysis with fluorescence detection of the culture material) 0, 50 or 150 mg FB_1 per kilogram diet for 5 weeks before mating and then after mating until embryonic day 16 at the conclusion of organogenesis. The doses were equal to 0, 8 and 25 mg/kg bw per day and 0, 13 and 39 mg/kg bw per day for the LM/Bc and CD-1 dams, respectively. Only one LM/Bc mouse in five litters in the 150 mg/kg diet group developed an NTD (exencephaly), and no NTDs were found in the CD-1 offspring, although elevated fetal death was observed in 20% of the litters in the 150 mg/kg diet group. Mild maternal hepatotoxicity was noted in the 150 mg/kg diet group from both strains. Analysis of the fetal livers (embryonic day 16) showed that sphinganine and sphinganine 1-phosphate were significantly elevated only in the LM/Bc strain consuming the 150 mg FB_1 per kilogram diet, consistent with the conclusion that dietary FB_1 can cross the mouse placental barrier, as shown in the study by Gelineau-Van Waes et al. (2005) using ^{14}C-labelled FB_1. The NOAELs for NTD induction and elevation in sphinganine and sphinganine 1-phosphate levels in fetal liver were 50 and 150 mg FB_1 per kilogram diet in LM/Bc and CD-1 mice, respectively (equal to 8 and 39 mg/kg bw per day) (Voss, Gelineau-Van Waes & Riley, 2006). In a follow-up study using the same protocol but doses of 0, 150 and 300 mg FB_1 per kilogram diet and only the LM/Bc strain, no NTDs were found (as reported in Voss, Riley & Gelineau-Van Waes, 2011). In the opinion of the Committee, the results of the second study make the setting of a NOAEL for NTD induction based on the results of the first study uncertain.

(ii) Rats

Pregnant Sprague-Dawley rats (200–210 g, 3 months old) were grouped into six treatment groups (10 animals per group) that were fed a control diet or diets containing 150 mg fumonisins per kilogram prepared from *F. verticillioides* culture material with or without either garlic extract or cabbage extract from embryonic days 6 to 15. Garlic extract control groups were fed the same diets but without fumonisins added. The fumonisin content of the diet was assessed using HPLC with fluorescence detection, but the levels of FB_1, FB_2 and FB_3 were not reported. Dams were killed on embryonic day 20 5 days after they were taken off the experimental diets. Even though the animals were removed from the diets 5 days before the toxicity assessment, there was clear evidence of maternal toxicity based on reduced body weight and feed intake. Fetal toxicity was also evident based on the number of dead fetuses, live fetuses, fetal body weight and skeletal malformations. The garlic treatment and, to a lesser extent, the cabbage treatment were effective in providing significant protection against both maternal and fetal toxicity. The authors concluded that fumonisin fetal toxicity was secondary to maternal toxicity and that, based on the lack of any significant elevation in the sphinganine to sphingosine ratio in the fetuses, fumonisin did not cross the placenta (Abdel-Wahhab et al., 2004). A NOAEL could not be determined by the Committee. The Committee was of the opinion that waiting 5 days after taking the animals off the experimental diets would have allowed for reversal of the effects on levels of sphingolipid metabolites in tissues and also possibly maternal liver and kidney toxicity, but that it would have had no effect on the fetal toxicity.

(iii) Rabbits

Crossbred (New Zealand × Chinchilla, 49 days old, 0.76 kg) male rabbits (12 animals per group) were fed diets prepared using *F. verticillioides* (MRC 826) culture material blended with a defined rabbit chow to achieve fumonisin concentrations (based on ELISA, Neogen) of 0.13, 5, 7.5 and 10 mg fumonisin per kilogram test diet for 196 days. The daily FB_1 exposure was reported to be equal to 0.005 (control diet), 0.199, 0.292 or 0.373 mg/kg bw. The concentrations of FB_2 and FB_3 were reported to have been negligible and not even detectable in most of the samples. The purpose of the study was to evaluate the effects of fumonisin on serum chemistry and haematological parameters at 84 days (Ewuola & Egbunike, 2008), organ weights and histopathology at 196 days (Ewuola, 2009) and reproductive effects at 175 and 196 days (Ewuola & Egbunike, 2010a,b). The toxicological aspects of this study that are not directly related to reproduction are presented in section 2.2.2. After 175 days on the diets, Ewuola & Egbunike (2010a) documented delayed onset of puberty and numerous effects indicative of impaired reproductive function, including decreased testicular weight, reduced sperm motility, increased sperm mortality and morphological abnormalities, and increased embryo mortality in does mated to bucks consuming the diets containing 7.5 mg fumonisin per kilogram and above. On day 196, gonadal and epididymal sperm reserves were significantly decreased in a dose-dependent manner at doses at and above 7.5 mg fumonisin per kilogram diet; some significant effects on sperm productivity were seen at doses at and above 5 mg fumonisin per kilogram diet (Ewuola & Egbunike, 2010b). At sacrifice on day 196, testes weights were significantly elevated at 7.5 mg

fumonisin per kilogram diet (Ewuola, 2009). Based on the histopathology scores, lesions in testes were judged "mild to moderate" in one of five, four of five and four of five animals at 5, 7.5 and 10 mg/kg, respectively. No lesion scored as "severe" at any dose level in testes. The effects on the various reproductive parameters occurred at levels equal to or greater than the doses that caused observable lesions in tissues (≤0.199 mg/kg bw) (see section 2.2.5). The authors concluded that dietary exposure to fumonisin at concentrations above 5 mg/kg (0.199 mg/kg bw per day) in male rabbits should be avoided, because it may reduce reproductive potential and efficiency (Ewuola & Egbunike, 2010a,b). A NOAEL could not be determined. In the opinion of the Committee, the accuracy of the fumonisin analysis of the diets is uncertain due to the use of ELISA. Nonetheless, the conclusion of the authors is consistent with the data.

(iv) Pigs

Male weanling pigs (White, 8–9 weeks old, 7 kg) were fed four diets (six per group) containing 0.2, 5, 10 or 15 mg FB_1 per kilogram prepared from *F. verticillioides* (MRC 826) culture material for 6 months. The level of fumonisin in the diet was determined using ELISA (Neogen). As reported in Gbore (2009a), the average daily dietary exposure to FB_1 was 0.2, 6, 11.5 and 17 mg/kg bw per day. The purpose of the study was to evaluate the effects of fumonisin on nutrient utilization (Gbore & Egbunike, 2007), growth performance (Gbore, 2009a), brain neurotransmitter levels (Gbore, 2010) and semen quality and sperm production (Gbore & Egbunike, 2008; Gbore, 2009b). The toxicological aspects of this study that are not directly related to reproduction are presented in section 2.2.2. In the pubertal boars, daily dry matter intake and feed conversion ratios were significantly increased (≥5 mg/kg), daily weight gain was decreased (≥10 mg/kg) and the onset of puberty was delayed (≥5 mg/kg). Statistically significant effects on sperm production (≥10 mg/kg diet) and semen quality (≥5 mg/kg diet) were found (Gbore & Egbunike, 2008, 2009b). The NOAEL in this study is that of the control group, 0.2 mg/kg bw per day. In the opinion of the Committee, an exact dose cannot be established due to the fact that the levels of fumonisins in the diets were based only on ELISA. Nonetheless, the results support the conclusion that consumption of the diets had significant effects on sperm production and quality.

(v) In vitro

ICR mouse embryos (3–5 somite stage) were cultured with 0, 1, 2, 3, 5, 7, 15, 25, 50 or 100 µmol FB_1 (90–95% pure) per litre with or without folinic acid for 26 hours (n = 10–36 per dose level). Other groups were exposed to 50 µmol FB_1 per litre for 2 hours followed by 24 hours in culture medium without FB_1 but with or without folinic acid (n = 18–25 per treatment). In the experiment with 2 hours of exposure to 50 µmol FB_1 per litre, the embryos were washed with fumonisin-free culture medium before being transferred to the culture bottles with or without folinic acid (1 mmol/l). The embryos grown in control medium with or without folinic acid supplementation grew normally and showed no malformations after 26 hours in culture (18–20 somite stage). Fumonisin exposure for 26 hours showed a significant dose-dependent increase in malformations (NTDs; exencephaly) and growth retardation at all FB_1 doses at and above 2 µmol/l. These effects occurred with only

minimal effects on somite development. Folinic acid supplementation significantly reduced the number of NTDs and growth retardation but did not reduce sphinganine accumulation in exposed embryos. In the experiment in which embryos were exposed for only 2 hours before transfer to fumonisin-free medium with or without folinic acid, the short-term exposure (2 hours) induced NTDs (67%), facial defects (83%) and growth retardation, all of which were significantly reduced by the post-exposure folinic acid treatment. In the short-term exposure experiment, the average somite numbers in the control, fumonisin-only treatment and the fumonisin followed by folinic acid supplementation treatment were 20.8, 18.3 and 18.5, respectively. The authors concluded that the fumonisin-induced NTDs were not a consequence of generalized embryotoxicity, but rather a specific effect on neural tube closure, as somite development was only minimally affected and was sufficient to have closed cranial neural folds (Sadler et al., 2002). The Committee concluded that the NOAEL in vitro for NTDs was 1 µmol FB_1 per litre.

2.2.6 Special studies

(a) Immunotoxicity

At the time of the 2001 evaluation, there were very few studies that directly addressed the potential for fumonisins to modify immune functions in vivo. Since then, more research has been done. The studies are described below, and in vivo studies are summarized in Table 7.

(i) In vivo experiments using pure/purified FB_1

Mice

To examine the effects of FB_1 on the immune system of BALB/c mice and to determine if there was sex specificity, female and male BALB/c mice (7–8 weeks of age and an average body weight of 20 g, five per group, acclimated for 1 week) received five daily subcutaneous injections of saline (vehicle control) or FB_1 at 2.25 mg/kg bw per day (endotoxin free; 100% purity, Promec). Fumonisin-free rodent chow (Harlan Teklad 22/5) and water were supplied ad libitum. Animals were sacrificed 24 hours after the final injection and were fasted for the final 12 hours; changes in body weight for the duration of treatment were calculated using the weight on day 5 of injection. Blood, thymus and spleen were collected for immunological examination. Several functional parameters were examined, including cytokine gene expression, lymphocyte blastogenesis and immune cell surface marker expression. Treatment with FB_1 resulted in a significant decrease in body weight gain in both female and male mice. FB_1 treatment reduced relative spleen and thymus weights in females only, whereas relative kidney (females only) and liver weights (in females more than in males) were increased. Total erythrocyte counts were increased only in male mice, whereas leukocyte counts were increased only in females. Circulating IgG concentrations were decreased in both sexes, but the decrease was greater in females. Exposure to FB_1 reduced splenic cellularity and the basal rate of lymphocyte proliferation in females only. In addition, phytohaemagglutinin P (PHA-P)-induced T-lymphocyte proliferation

Table 7. Overview of in vivo studies on immunotoxicity of fumonisins

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Using pure/purified FB_1										
Female and male BALB/c mice (7–8 weeks old, average 20 g)	FB_1 (100%, Promec)	5 days, sacrificed 24 h after the final injection	5	—	0 or 2.25	Subcutaneous	*Males:* Decreased body weight gain Increased relative liver weight Increased total erythrocyte counts Decreased circulating IgG concentrations No changes in the thymocyte populations *Females:* Decreased body weight gain Reduced relative spleen and thymus weights Increased relative kidney weight Increased relative liver weight (more than in males) Increased leukocyte counts Decreased circulating IgG concentrations (more than in males)	2.25	<2.25	Johnson & Sharma (2001); Johnson et al. (2001)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
							Reduced splenic cellularity and the basal rate of lymphocyte proliferation Reduced PHA-P-induced T-lymphocyte and LPS-induced B-lymphocyte proliferation Reduced expression of IL-2 mRNA of splenocytes No alterations in TNFα or IL-1β mRNA expression Relative increase in the T-lymphocyte population in the spleen Dramatic reduction in immature CD4+/CD8+ double positive cell population in thymus			

Table 7 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Male Wistar rats (6–8 weeks old)	Purified FB_1 (as described in Voss et al., 1990)	12 weeks	6	<0.3 or 100	0 or 5	Diet	Reduced feed consumption, body weight, and body weight gain (day 90) Histiocytic perivascular infiltrate and an increased number of Kupffer cells in the liver Necrosis and apoptosis of tubular epithelial cells in the kidney Increased mitotic figures and lymphocytic infiltrate in the small intestine Elevated serum enzyme alkaline phosphatase Decreased triglyceride levels No effect on mitogen-induced proliferation of spleen mononuclear cells Decreased H_2O_2 release by peritoneal macrophages No changes in production of superoxide anion by total peritoneal cells	5	<5	Theumer et al. (2002)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Male weaned Yorkshire hybrid pigs (3 weeks old)	Purified FB_1 (Promec)	7 days	8 (control) or 9 (treatment)	—	0 or 0.5	Gavage	No effect on IL-1β, IL-6, IL-12 and TNFβ mRNA levels in ileum Downregulation of IL-8 mRNA in the pig ilea, confirmed in vitro in porcine intestinal epithelial cell line IPEC-1	0.5	<0.5	Bouhet et al. (2006)
Weanling piglets (7.3 ± 0.4 kg)	Purified FB_1 (>98%)	7 days	6	—	0 or 1.5	Gavage	Decrease in IL-4 and increase in IFN-γ expression in ex vivo PHA-stimulated blood cells	1.5	<1.5	Taranu et al. (2005)
Using culture material										
Female or castrated male crossbred piglets (4 weeks old)	Feed with *F. verticillioides* culture material; 54% FB_1, 8% FB_2 and 9% FB_3	28 days, immunized subcutaneously with a *Mycoplasma agalactiae* vaccine on day 7 and day 21	5	0 or 8	Equivalent to 0 or 0.32	Diet	*Males:* Decreased body weight gain Higher level of creatinine Decreased specific antibody levels after vaccination Decreased mRNA expression level of IL-10 *Females:* Higher level of creatinine No effect on specific antibodies or on cytokine mRNA levels	0.32	<0.32	Marin et al. (2006)

Table 7 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Weanling crossbred pigs (12.3 ± 0.8 kg) (sex unknown)	Fumonisin extract *F. verticillioides* strain NRRL 34281 in a corn soya bean meal basal diet (54% FB_1, 8% FB_2 and 9% FB_3)	28 days; at days 8 and 22, subcutaneous immunization with Agavac, a vaccine made with a combination of formol-inactivated *Mycoplasma agalactiae* strains	20	0 or 8	Equivalent to 0 or 0.32	Diet	Decreased expression of IL-4 mRNA by porcine whole blood cells Diminished specific antibody titre against *M. agalactiae* No effect on serum concentration of the immunoglobulin subset (IgG, IgA and IgM)	0.32	<0.32	Taranu et al. (2005)
Weaned castrated male piglets (age unknown, 12–14 kg)	FB_1 from *F. moniliforme* culture	3–4 months	5	0, 1, 5 or 10	Equivalent to 0, 0.04, 0.2 or 0.4	Diet	No effect on specific and nonspecific immune response (specific antibody titre, PHA-, ConA- or LPS-induced lymphocyte proliferation)	—	0.4	Tornyos et al. (2003)
		8 days	6 (control) 14 (dose)	Unknown	Equivalent to 0 or 7–8, using reported body weights of 12–14 kg	Diet		—	7	

ConA, concanavalin A; IFN, interferon; IL, interleukin; LPS, lipopolysaccharide; mRNA, messenger ribonucleic acid; PHA, phytohaemagglutinin; PHA-P, phytohaemagglutinin P; TNF, tumour necrosis factor

and lipopolysaccharide (LPS)-induced B-lymphocyte proliferation were reduced in female mice. Splenocytes from female mice exposed to FB_1 showed a reduced expression of interleukin-2 (IL-2) messenger ribonucleic acid (mRNA). These changes occurred in the absence of alterations in TNFα or IL-1β mRNA expression. Phenotypic analysis indicated that FB_1 treatment caused a relative increase in the T-lymphocyte population in the spleen of female mice only. In contrast, FB_1 dramatically reduced the immature CD4+/CD8+ double positive cell population in the thymus of females. No changes were evident in the thymocyte populations of male mice treated with FB_1. The authors concluded that FB_1 is immunosuppressive in mice and that the effect is sex dependent, with females being more susceptible than males (Johnson & Sharma, 2001; Johnson et al., 2001). As the exposure to FB_1 was performed by subcutaneous injection, the Committee could not derive an oral NOAEL for immunotoxicity from this study.

Rats

Groups of six male 6- to 8-week-old Wistar rats were exposed to 0 (control, FB_1 <0.3 mg/kg diet) or 100 mg purified FB_1 (as described in Voss et al., 1990) per kilogram diet (equivalent to 0 or 5 mg/kg bw per day) for 12 weeks. The FB_1 content of the diet was checked with HPLC. Feed consumption, body weight, body weight gain and fumonisin consumption were determined. Specimens of lungs, spleen, liver, kidney and small intestine were obtained for examination by light microscopy. The levels of total cholesterol, triglycerides and calcium and the enzymatic activities of AST, ALT, γ-glutamyl transferase (GGT) and alkaline phosphatase in serum obtained from intracardiac puncture were determined using a Technicon RA-1000 autoanalyser. Mitogenic responses of spleen mononuclear cells to concanavalin A (ConA) and LPS were determined (in vivo as well as in vitro with 10 µmol/l FB_1), as well as cytokine levels in supernatants of spleen mononuclear cells after 24 hours (IL-2) and 72 hours (IL-4, IL-10) of culture. The levels of hydrogen peroxide produced by adherent peritoneal cells and the levels of anion superoxide produced by total peritoneal cells in the basal state and in the presence of phorbol myristate acetate (PMA) were quantified.

Feed consumption, body weight and body weight gain on day 90 were reduced in animals exposed to FB_1. The total FB_1 exposure on day 90 was 810 mg/kg bw. Histopathological changes in tissues of FB_1-exposed animals consisted of histiocytic perivascular infiltrate and an increased number of Kupffer cells in the liver, necrosis and apoptosis of tubular epithelial cells in the kidney, and increased mitotic figures and lymphocytic infiltrate in the small intestine. Serum alkaline phosphatase activity was significantly elevated in rats fed FB_1, whereas triglyceride levels were decreased compared with controls. Treatment with FB_1 in vivo or in vitro did not have a significant effect on mitogen-induced proliferation of spleen mononuclear cells; the authors stated that this was due to the low concentration of FB_1. However, increased levels of IL-4 and decreased levels of IL-10 were released by treated cells in culture compared with controls. FB_1 in vivo or in vitro decreased the hydrogen peroxide released by peritoneal macrophages, whereas no changes in levels of superoxide anion produced by total peritoneal cells were detected. The authors concluded that subchronic FB_1 exposure could affect the small intestine and alter the interleukin profile and some main functions of macrophages in antitumour

activity (Theumer et al., 2002). As effects were seen at the only dose tested, the Committee could not derive a NOAEL from this study.

Pigs

Seventeen 3-week-old weaned healthy male Yorkshire hybrid pigs received 0 (eight animals) or 0.5 mg (nine animals) of purified FB_1 (Promec, in sterile water) per kilogram of body weight per day by gavage for 7 days. The purpose of the study was to determine the effects of FB_1 on intestinal immune response. After necropsy, ileal samples were analysed for mRNA expression for five pro-inflammatory cytokines by reverse transcriptase polymerase chain reaction (RT-PCR). No difference was observed for IL-1β, IL-6, IL-12 or TNFβ mRNA levels between control and FB_1-treated animals. In contrast, FB_1 treatment induced a significant downregulation of the expression of IL-8 mRNA in the pig ilea. The effect of FB_1 on IL-8 expression was also examined in the porcine intestinal epithelial cell line IPEC-1. FB_1 decreased the expression of IL-8, at both the mRNA and protein levels, in a dose-dependent manner. The authors concluded that FB_1 alters the intestinal immune response by decreasing the level of IL-8 (Bouhet et al., 2006). As effects were seen at the only dose tested, the Committee could not derive a NOAEL from this study.

Two groups of six weanling piglets with an initial average body weight of 7.3 ± 0.4 kg were given by gavage 0 or 1.5 mg/kg bw per day of purified FB_1 (>98% pure by nuclear magnetic resonance [NMR], MS and HPLC) diluted in water for 7 days. Control animals received 4 ml of sterile water. At the end of the experiment, piglets were sacrificed; blood samples were collected to measure cytokine mRNA expression upon in vitro stimulation, as well as spleen and mesenteric lymph node tissue samples. After in vitro stimulation with phytohaemagglutinin (PHA), porcine blood cells expressed mRNA encoding for interferon-gamma (IFN-γ) and IL-4, but the cytokine expression pattern was altered in tissue samples from FB_1-exposed animals, with IL-4 expression lower and IFN-γ higher than controls (Taranu et al., 2005). As effects were seen at the only dose tested, the Committee could not derive a NOAEL from this study.

(ii) In vivo experiments using culture material

Pigs

Groups of five female or castrated male crossbred piglets (4 weeks old, acclimatized for 1 week) received for 28 days either control feed (maize–soya bean meal-based diet) or feed contaminated with 8 mg FB_1 per kilogram feed (equivalent to 0.32 mg/kg bw per day) in the form of *F. verticillioides* culture material. The crude extract contained 54% FB_1, 8% FB_2 and 9% FB_3. At day 7 and day 21, animals were immunized subcutaneously with a *Mycoplasma agalactiae* vaccine. Body weights and feed consumption were recorded weekly throughout the experiment. On day 0, day 20 and day 28 of the experiment, blood samples were aseptically collected by jugular vein puncture. Serum concentrations of sodium, potassium, chloride, calcium, phosphorus, total proteins, urea, creatinine, glucose, cholesterol, triacylglycerols and bilirubin and activities of alkaline phosphatase, AST, ALT, GGT and lactate dehydrogenase (LDH) were determined. Total concentrations of the

different immunoglobulin subsets (IgG, IgA and IgM) and antibodies against *M. agalactiae* were measured by ELISA. The mRNA expression for five different cytokines was analysed in the blood samples by semiquantitative RT-PCR.

Ingestion of FB_1-contaminated feed significantly decreased body weight gain in males but had no effect in females. The reduced weight gain was not due to reduced feed intake. A statistically significantly higher level of creatinine was noted in all toxin-treated animals. The specific antibody production against *M. agalactiae* was increased in both males and females; on day 28, however, the increase in specific antibody production in the fumonisin-fed males was significantly less than in the control group, whereas in females, there was no difference in the specific antibody production compared with the female control group. Males, but not females, showed a significant reduction in expression of IL-10, and other T helper 2 (Th2) cytokines were decreased, but not significantly. The authors concluded that FB_1 is immunosuppressive in pigs and that the magnitude of the effect is highly dependent on sex, with males being more susceptible than females (Marin et al., 2006). The Committee noted that the results also indicated a non-statistically significant decrease in GGT, AST and LDH activities and serum IgA concentrations in treated males. As effects were seen at the only dose tested and as other fumonisins were present in the feed, the Committee could not derive a NOAEL for FB_1 from this study.

Groups of 20 weanling crossbred piglets with an initial average body weight of 12.3 ± 0.8 kg were exposed to fumonisin extract in a corn–soya bean meal basal diet for 28 days. The fumonisin extract was obtained after in vitro culture of the *F. verticillioides* strain NRRL 34281 (Oswald et al., 2003) and was incorporated into the diet to provide a feed diet containing 8 mg FB_1 per kilogram (equivalent to 0.32 mg/kg bw per day). The crude extract contained 54% FB_1, 8% FB_2 and 9% FB_3 (see the above-described study of Marin et al., 2006). At days 8 and 22 of the experiment, animals were immunized subcutaneously with 1 ml of Agavac, a vaccine made with a combination of formol-inactivated *Mycoplasma agalactiae* strains (Pasteur Institute, Bucharest, Romania). Blood samples were obtained throughout the experiment to measure total and specific antibody levels as well as cytokine mRNA expression upon mitogenic stimulation. Exposure to the contaminated diet resulted in a statistically significant decrease in the expression of IL-4 mRNA by porcine whole blood cells and diminished the specific antibody titre after vaccination against *M. agalactiae*. By contrast, ingestion of the contaminated feed had no effect on the serum concentration of the immunoglobulin subset (IgG, IgA and IgM). The authors concluded from the combined results (with in vitro and 7-day study described under experiments with pure FB_1) that FB_1 alters the cytokine profile and decreases the specific antibody response built during a vaccination protocol (Taranu et al., 2005). As effects were seen at the only dose tested and as other fumonisins were present in the feed, the Committee could not derive a NOAEL for FB_1 from this study.

Fusarium moniliforme (strain unknown) fungal culture was added to the diet of weaned castrated piglets of identical genotype (breed unknown, 12–14 kg) to ensure an FB_1 exposure of 0, 1, 5 or 10 mg/kg diet (first experiment, equivalent to 0, 0.04, 0.2 or 0.4 mg/kg bw per day for 3–4 months, five animals per group) or 0 or 100 mg per animal per day for 8 days (second experiment, equivalent to

approximately 7–8 mg/kg bw per day based on reported body weight, 6 animals in control group, 14 animals in test group). In order to determine the immune response, the animals were vaccinated against Aujeszky disease with inactivated vaccine (Aujespig K, Phylaxia-Sanofi, Budapest, Hungary). Specific and nonspecific in vitro cellular immune responses were measured by the lymphocyte stimulation test induced by PHA-P, ConA, LPS and inactivated suspension of the Aujeszky disease virus. Humoral immune response (e.g. specific antibody titre) was measured by the virus neutralization test. None of the immunological parameters examined showed significant differences between groups, but in the second experiment, animals died within 0.5–1 day of onset of rapid and severe pulmonary oedema after 5–7 days of dosing. The authors concluded that FB_1 had no significant effect on the humoral or cellular specific and nonspecific immune responses when fed in a very high dose (approximately 7–8 mg/kg bw per day for 8 days) or in a low dose for even a longer period (0.04, 0.2 or 0.4 mg/kg bw per day for 3–4 months) (Tornyos et al., 2003).

(iii) In vitro experiments using pure/purified fumonisin B_1

Porcine cells

The influence of FB_1 on the in vitro production of IFN-γ and IL-4 was tested in porcine peripheral blood mononuclear cells. Peripheral blood mononuclear cells cultured at a density of 5×10^6 cells per well and stimulated with 10 µg/ml of ConA were incubated with increasing concentrations of FB_1 (0, 2, 5, 10, 20, 50 or 100 µmol/l, >98% pure by NMR and HPLC) for 96 hours. Culture supernatants were collected, and concentrations of IFN-γ and IL-4 cytokine were measured by ELISA. Results from four independent experiments indicated that FB_1 decreased IL-4 and increased IFN-γ synthesis at both the protein and mRNA levels, at concentrations of 10 µmol/l and higher (Taranu et al., 2005).

Swine peripheral blood mononuclear cells were incubated with purified FB_1 (>98% pure by NMR and HPLC, toxin dissolved in water and further diluted in complete cell culture medium) at concentrations of 0, 10, 50, 100 or 200 µmol/l for 48 hours. After incubation, effects on cell proliferation, cell cycle progression and IL-2 production were evaluated. FB_1 induced a decrease of ConA-induced peripheral blood mononuclear cell proliferation as measured by cell counting and dehydrogenase enzyme activity. This effect was observed starting with an FB_1 concentration of 10 µmol/l. The effect of FB_1 on cell cycle progression was analysed by flow cytometry. Incubation of peripheral blood mononuclear cells with increasing concentrations of FB_1 increased the percentage of cells blocked in G0/G1 phase of the cell cycle. Treatment with FB_1 at 200 µmol/l induced a high blockade of the cell cycle, with 92.4% of cells in G0/G1 phase, almost the percentage of unstimulated cells, which was 95.8%. This blockade was observed in all lymphocyte subsets tested (CD2+, CD4+, CD8+ and Ig+), as evidenced by dual staining for DNA and membrane surface molecules. A significant decrease in IL-2 production was also observed in the supernatants of ConA-stimulated peripheral blood mononuclear cells treated with FB_1 at 100 or 200 µmol/l, whereas at 5 and 10 µmol/l, IL-2 production was increased. Based on these results, the authors proposed that FB_1 may affect immune functions by inhibiting lymphocyte proliferation and IL-2

production, suggesting a possible role of FB_1 exposure in infectious disease and cancer (Marin et al., 2007).

Human and porcine cells

The independent effect of purified FB_1 (>98% pure by NMR and HPLC) on lymphocyte proliferation using human and porcine lymphocytes was investigated, as well as of AFB_1, deoxynivalenol (DON) and nivalenol (NIV). Human and porcine peripheral blood mononuclear cells and porcine splenocytes were cultured, stimulated with PMA and ionomycin and incubated with each mycotoxin at concentrations ranging from 0.001 to 1000 µg/ml (at log intervals) for 72 hours and labelled in the previous 24 hours with [methyl-^{3}H]thymidine. The results showed that increasing concentrations of AFB_1, DON and NIV affected the [methyl-^{3}H]-thymidine cellular proliferation following mitogen stimulation in both species and cell types. Lower concentrations of mycotoxins (up to 0.1 µg/ml) enhanced cellular proliferation, which was more pronounced in human than in porcine cells, whereas higher concentrations (1–10 µg/ml) caused a dose-dependent decrease for both types of lymphocytes and for both species. NIV was the most potent mycotoxin in both species and both cell types, with median inhibitory concentrations (IC_{50}s) of 0.05 µg/ml (porcine splenocytes), 0.09 µg/ml (porcine peripheral blood mononuclear cells) and 0.08 µg/ml (human peripheral blood mononuclear cells). FB_1 was the least potent, with IC_{50}s of 13.5 µg/ml (porcine splenocytes), 650 µg/ml (porcine peripheral blood mononuclear cells) and 163 µg/ml (human peripheral blood mononuclear cells) (Taranu et al., 2010).

Human cells

To determine and compare the morphological effects of FB_1 and ochratoxin A (OTA) on lymphocytes and neutrophils harvested from the circulation of healthy volunteer subjects and patients with oesophageal and breast carcinomas, these lymphocytes and neutrophils were incubated with FB_1 and OTA (Sigma, purity unknown). The incubation was performed at the previously determined IC_{50} (50% survival/death of leukocytes) concentration and time of 25 µg/ml (FB_1) for 2 hours in lymphocytes and 50 µg/ml (OTA) for 23 hours in neutrophils. After incubation, viability of leukocytes (3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide) [MTT] test), morphology of leukocytes (transmission electron microscopy), circulating cytokine levels (ELISA) and cytokine release from leukocytes (ELISA) were measured. Also, chemokine, granulocyte-colony simulating factor and TNFα receptor localization on the leukocytes were determined by immunolabelling.

The authors reported that both FB_1 and OTA reduced the number of viable lymphocytes and neutrophils harvested from the circulation of volunteer subjects and carcinoma patients in a dose-dependent manner, but data on different doses were not presented. The neutrophils showed disrupted cell membranes, damage to cytoplasmic organelles and loss of nuclear integrity; however, these effects were not quantified. The authors suggested that FB_1 and OTA have an immunosuppressive effect in humans, in particular patients with cancer, by impairing immune surveillance (Odhav et al., 2008).

Immunotoxic effects of FB_1 exposure were examined in human dendritic cells differentiated from human peripheral blood mononuclear cells. Messenger RNA and protein levels of a number of cytokines and chemokines were analysed in dendritic cells, after exposure to FB_1 (Sigma, purity unknown) at 100 µmol/l, LPS at 10 ng/ml or a combination of the two for 6 hours or 24 hours. Exposure to FB_1 resulted in an increase in the expression of IFN-γ and CXCL9. FB_1 inhibited the LPS-induced expression of IL-6, IL-1β, CCL3 and CCL5. The other cytokines studied (TNFα, IL-12, IL-18 and IL-23) were not affected by FB_1 in dendritic cells (Stockmann-Juvala et al., 2008).

(b) Neurotoxicity

In the 2001 evaluation, ELEM, now recognized to be the result of cardiovascular toxicity, was the main neurotoxic effect known to result from fumonisin exposure. Additional studies in different species have been performed since then. Because of their design (intraperitoneal exposure, single dose group), these studies were deemed not suitable for providing a point of departure for the evaluation. As they do provide new insights in the neurotoxicity of fumonisins, they are taken up in this monograph addendum. All studies are described below, and those studies conducted by the oral route of administration are summarized in Table 8.

(i) Mice

The toxicity of FB_1 in brain after an intracerebroventricular or subcutaneous infusion was compared using 7- to 8-week-old female BALB/c mice (five animals per group, Harlan). Animals were infused (0.5 µl/h) with total doses of 0, 10 or 100 µg FB_1 (98% purity; Promec) in saline over 7 days via osmotic pumps implanted either via intracerebroventricular cannulation of the ventricle or via the subcutaneous route. One day after the last day of treatment, brains were dissected either fresh or after intracardiac paraformaldehyde fixation. Intracerebroventricular exposure of mice to FB_1 resulted in reduced weight gain only in animals treated with the higher (100 mg) amount of FB_1; brain weights remained unaffected in all treatment groups. The condition of intracerebroventricularly exposed animals to 100 mg of FB_1 deteriorated progressively during the treatment. Mice developed locomotor problems, such as shaky gait and tremors; spasticity of extremities occurred towards the end of the treatment period. In these mice, there was neurodegeneration in the cortex, and astrocytes were activated in the hippocampus. HPLC indicated accumulation of free sphinganine in animals given FB_1 intracerebroventricularly in all brain regions and increased free sphingosine in the cortex after the 100 µg FB_1 dose. Free sphingosine showed a non-significant increasing trend for the medulla oblongata. The concentrations of cortical sphingomyelin and complex sphingolipids remained unchanged. The intracerebroventricular administration of FB_1 induced expression of TNFα, IL-1β, IL-6 and IFN-γ after both doses, assayed by the real-time PCR. The subcutaneous administration of 100 µg FB_1 caused slight sphinganine accumulation and increased IL-1β expression in cortex only. The Committee concluded that these sphinganine changes could not be definitively attributed to FB_1 crossing the blood–brain barrier, as they occurred at doses of FB_1 causing large elevations in ALT and AST activities and other signs of marked toxicity. The authors concluded that

Table 8. In vivo oral studies on neurotoxicity

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Pure/purified FB_1										
Two pregnant rats (breed unknown)	FB_1 (purity unknown)	From gestation day 8 until parturition	5 pups per treated dam	—	0 or 6.2	Gavage	Induction of long-term potentiation, which was seen as an elementary learning phenomenon, was enhanced by 71–127%, but no data presented Evoked response from single stimuli before and 30 min after tetanic stimulation Longer duration, ictal type of epileptiform seizure activity was seen in response to induction with 4-AP (epilepsy model) Increased latency and amplitude of spontaneous seizures in 50 µmol/l 4-AP solution Decreased frequency of spontaneous seizures in 50 µmol/l 4-AP solution	6.2	<6.2 *Owing to the lack of detail, for instance on condition of the dams and pups, the Committee does not consider these two studies fit to be used in the evaluation*	Banczerowski et al. (2008); Banczerowski-Pelyhe et al. (2008)

Table 8 (contd)

Species description	Compound (purity)	Length of study	No. per group	Dose (mg/kg diet)	Dose (mg/kg bw per day)	Route	Effect	LOAEL (mg/kg bw per day)	NOAEL (mg/kg bw per day)	Reference
Male weanling pigs (White, 8–9 weeks old, 7 kg)	FB_1 from *F. verticillioides* (MRC 826) culture material	6 months	6	0, 0.2, 5, 10 or 15	Reported to be equal to 0, 0.2, 6, 11.5 or 17	Diet	Significantly decreased specific acetylcholinesterase activity in the amygdala Significant increases in specific acetylcholinesterase activity in the hippocampus and hypothalamus	6	0.2 *Owing to the poorly defined dietary doses, discrepancies in the reported doses and lack of a clear dose–response relationship, the Committee concluded that these effects on acetylcholinesterase activity could not be attributed to* FB_1 *exposure*	Gbore (2010)

4-AP, 4-aminopyridine

intracerebroventricular injection of FB_1 caused neurodegeneration with simultaneous inhibition of de novo ceramide synthesis, stimulation of astrocytes and upregulation of pro-inflammatory cytokines in the murine brain and that a relative lack of FB_1 availability in the brain could be responsible for the absence of its neurotoxicity in mice (Osuchowski, Edwards & Sharma, 2005).

Groups of five (control, FB_1 and LPS groups) or nine (FB_1 + LPS group) 7- to 8-week-old BALB/c mice (Harlan, acclimatized for 1 week) were injected intraperitoneally with LPS (3 mg/kg) or phosphate-buffered saline at the beginning of treatment, followed by subcutaneous injection of FB_1 (98% purity, 2.25 mg/kg) either as a single injection (2 hours after the LPS) or in three daily doses (6, 24 and 48 hours after LPS). Control animals received phosphate-buffered saline via the respective route. Based on the feed consumed by mice, the FB_1 dose was calculated by the authors to be equal to 14 mg/kg in diet (equivalent to 2.1 mg/kg bw per day). Mice were decapitated 4 hours after the single FB_1 injection or 24 hours following the last FB_1 injection for repeated FB_1 treatment. Blood was collected for analysis of ALT and AST. Livers and brains were analysed for free sphingosine and sphinganine, cytokine gene expression and liver cell proliferation. Sodium fluorescein (200 µl of 0.5%) or a similar volume of phosphate-buffered saline was injected intravenously 1 hour before sampling the animals (n = 4) to assess blood–brain barrier permeability.

In liver, FB_1 injection increased sphinganine levels in both treatments, but sphingosine levels after a single dose only. LPS did not have any effect on sphinganine levels, but increased sphingosine levels. Circulating ALT was increased by LPS alone after the single dose, whereas after the repeated doses, FB_1 induced ALT and AST, which was reduced by LPS treatment. The expression of TNFα, IFN-γ and IL-1β was increased after a single injection but not after repeated FB_1 treatment. LPS increased the expression of TNFα and IL-1β, but not of IFN-γ, in both dosing regimes. LPS decreased the effect of FB_1 on TNFα, IFN-γ after single treatment and IFN-γ only after repeated treatment. In brain, no effect of FB_1 was observed on the expression of TNFα, IFN-γ, IL-1β, IL-6 and IL-12, whereas LPS increased all. Repeated FB_1 treatment increased accumulation of sphinganine, which was enhanced by LPS treatment. FB_1 decreased the LPS-induced brain expression of IFN-γ and IL-1β, whereas the expression of IL-6 and IL-12 was enhanced (Osuchowski, He & Sharma, 2005).

(ii) Rats

Two pregnant rats received 0 or 6.2 mg/kg bw per day of FB_1 (purity unknown) by gavage (solvent unknown) from gestation day 8 until parturition. Per treated dam, five pups 4 weeks of age were sacrificed, and 400 µm thick transverse slices were prepared from the somatosensory cortex for ex vivo electrophysiological recording. When responses were stabilized after determining the stimulus strength evoked response curve (input–output curve), long-term potentiation was induced by high-frequency stimulation at 2T stimulus intensity (100 Hz, 4 × 5 seconds, 15-second intervals). Evoked responses were tested using single stimuli before and 30 minutes after tetanic stimulation (unknown method), and input–output curves were determined. Averaged latency and peak to peak amplitude of each component of

the evoked response were determined. The authors stated that the induction of long-term potentiation, which was seen as an elementary learning phenomenon, was enhanced by 71–127%, but no data were presented. In control animals, the amplitude of evoked field potentials at 2T stimulation was reported to increase from 0.612 to 0.836 mV by tetanic stimulation. The amplitude of evoked field potentials at 2T stimulation in offspring of treated dams was reported to be significantly higher, before and after long-term potentiation induction; however, statistical analysis of the raw data was not shown. The authors claimed that this basic neuronal excitability was FB_1 induced and that it may underlie modification of learning and memory processes (Banczerowski-Pelyhe et al., 2008).

In an experiment using the same exposure protocol, averaged latency and peak to peak amplitude of each component of the response were determined after seizure activity was induced by 4-aminopyridine (4-AP) (50 µmol/l, after recording of control values) as an epilepsy model. In the slices from the offspring of FB_1-treated pregnant rats, a longer-duration, ictal type of epileptiform seizure activity was seen. The latency and amplitude of the spontaneous seizures in 50 µmol/l 4-AP solution were significantly increased, whereas the frequency was decreased. The authors stated that, although the latency increased, the sensibility was also increased (Banczerowski et al., 2008). Owing to the lack of detail, for instance on condition of the dams and pups, the Committee did not consider these two studies fit to be used in the evaluation.

(iii) Pigs

In studies described in section 2.2.2(b), male weanling pigs (White, 8–9 weeks of age, 7 kg) were fed diets (six per group) containing FB_1 prepared from *F. verticillioides* (MRC 826) culture material for 6 months, where the level of fumonisin in the diet was determined using ELISA (Neogen). As reported in Gbore (2009a), the average daily dietary exposure to FB_1 was 0.2, 6, 11.5 and 17 mg/kg bw. The effects of fumonisin on brain neurotransmitter levels were evaluated.

Analysis of brain acetylcholinesterase activity normalized to protein content showed significantly decreased specific activity in the amygdala (≥5 mg/kg diet), medulla oblongata (5 mg/kg diet) and cerebral cortex and mid-brain (5 and 15 mg/kg diet, but not 10 mg/kg diet, for both). Significant increases were reported in the hippocampus and hypothalamus (≥5 mg/kg) (Gbore, 2010). The Committee concluded that, owing to the poorly defined dietary doses, discrepancies in the reported doses and lack of a clear dose–response relationship, these effects on acetylcholinesterase activity could not be attributed to FB_1 exposure.

(iv) In vitro experiments

Neurotoxic effects of FB_1 exposure were examined in human glioblastoma (U-118MG) and neuroblastoma (SH-SY5Y) cell lines. The production of reactive oxygen species, lipid peroxidation, intracellular reduced glutathione levels, cell viability, caspase-3-like protease activity and DNA fragmentation were studied after exposure to FB_1 (Sigma) at 0.01–100 µmol/l for 0.5–144 hours. FB_1 increased lipid peroxidation and the production of reactive oxygen species in U-118MG cells, showing significant effects after culture times from 48 to 144 hours at dose levels of

10 or 100 µmol/l. These effects were accompanied by decreases in the glutathione levels and cell viability after incubating the cells for 48–144 hours with the toxin. Signs of apoptosis were indicated by increased caspase-3-like protease activity and internucleosomal DNA fragmentation. Thus, oxidative stress and apoptosis may be involved in the neurotoxicity induced by FB_1 (Stockmann-Juvala et al., 2004a).

Similar results indicating oxidative stress were found in human neuroblastoma (SH-SY5Y) rat C6 glioblastoma and mouse GT1-7 hypothalamic cells, with the exception that no production of reactive oxygen species was seen in the SH-SY5Y cell line. Caspase-3 activity was not measured in this study (Stockmann-Juvala et al., 2004b).

Using the same experimental set-up, the role of apoptosis in the neurotoxicity of FB_1 was further explored by testing the activation of caspase-3-like protease, DNA fragmentation and expression of p53 and Bcl-2 family proteins. Caspase-3-like protease activity increased in all cell lines, except SH-SY5Y, at 48–144 hours, and internucleosomal DNA fragmentation occurred in all of the cell lines; however, the expressions of p53 or pro-apoptotic or antiapoptotic Bcl-2 family proteins (Bax, Bcl-2, Bcl-X_L and Mcl-1) were not affected in any of the cell lines, even after prolonged exposure to FB_1 at high doses (Stockmann-Juvala et al., 2006).

The authors hypothesized that caspase-3 activation could be one of the triggers that lead to increased production of reactive oxygen species, lipid peroxidation and decreased intracellular glutathione levels prior to DNA fragmentation and cell death, contrary to the belief that apoptosis and caspase activation are a consequence of oxidative stress due to production of reactive oxygen species, subsequent lipid peroxidation and reduced levels of glutathione. They indicated that both necrosis and apoptosis have a role in FB_1-induced neurotoxicity and that the sensitivities of the cell lines seemed to be human U-118MG glioblastoma cells > mouse GT1-7 hypothalamic cells > rat C6 glioblastoma cells > human SH-SY5Y neuroblastoma cells.

These results are in line with the observations of Osuchowksi & Sharma (2005), who also found that primary and established murine brain glial cells were vulnerable to FB_1-dependent cytotoxicity in vitro, whereas neuronal cells were not. FB_1 induced necrotic cell death and downregulation of proinflammatory signalling (TNFα and IL-1β) in both a microglial (BV-2) cell line and primary astrocytes and exhibited potent antiproliferative properties in BV-2 cells only. No effects were found on a neuroblastoma (N2A) cell line or primary cortical neurons other than accumulation of free sphinganine and decreased levels of free sphingosine, which were found in all four tested cell types.

(c) Combined toxicity of fumonisins with other mycotoxins

The possible combined toxicity of fumonisins with other mycotoxins was not considered in the previous evaluation. Since then, the interactions in vitro and in vivo between fumonisin and other mycotoxins—i.e. AFB_1, DON, zearalenone (ZEA), OTA, penicillic acid (PA), citrinin (CIT), moniliformin (MON) and beauvericin (BEA)—have been investigated in a large number of studies. The studies are

summarized below; studies testing the interaction of fumonisins with royal jelly, garlic and cabbage seed extract, and rooibos tea were not included.

(i) Fumonisin B_1 and other fumonisins

Chickens

FB_1, FB_2 and FB_3, individually and in combination (3:1:1 ratio), were injected into the air cell of chicken eggs at 72 hours of incubation. The mycotoxins caused haemorrhages of the head, neck and thorax, FB_1 being the most toxic. The toxicity of FB_1 alone was higher than that of the combined treatment (Henry & Wyatt, 2001).

(ii) Fumonisin and aflatoxin

Rats

The acute toxicity of combined oral administration of different doses of AFB_1 and FB_1 (>98% purity) was tested in male Fischer 344 rats. The median lethal dose (LD_{50}) of AFB_1 alone was 2.71 mg/kg bw. For FB_1 alone, the LD_{50} was greater than 46.4 mg/kg bw. Combined exposure to various doses of AFB_1 and a fixed FB_1 dose of 25 mg/kg bw yielded an LD_{50} of 1.37 mg/kg bw (McKean et al., 2006).

In a 90-day study, the nutritional alterations and immunobiological effects in rats fed control feed or feed containing AFB_1 at 40 µg/kg or a mixture containing AFB_1 at 40 µg/kg and FB_1 at 100 mg/kg were investigated in male Wistar rats. The Committee noted that descriptions of the methods and results were not consistent between the different sections of the paper. The descriptions of the methods are unclear and incomplete, and concentrations of biochemical parameters were not given. The Committee considered that the study was unsuitable (Theumer et al., 2003).

In a 90-day feeding study, male Wistar rats (six per group) received diets containing fumonisins prepared using *F. verticillioides* culture material (100 mg/kg, equal to 9.7–10.4 mg/kg bw per day of FB_1, FB_2 and FB_3 in a 4.46:1.00:2.03 ratio), AFB_1 at 40 µg/kg (equal to 4.1–4.2 µg/kg bw per day, AFB_1 purity >98%) or a mixture of the mycotoxins.

Absolute body weight and feed consumption were decreased after exposure to FB_1 only and exposure to the mixture. AFB_1, alone or with FB_1, induced thickened alveolar walls and lymphocytic infiltrates in the lung. In the mixture group, mitotic cells (liver), hepatocytes and kidney tubular cells with apoptosis morphology and lymphocytic infiltrates in the small intestine were observed. Tubular apoptotic cells and lymphocytic infiltrates in the small intestine were observed in the FB_1 group. Apoptosis, as observed with the terminal deoxynucleotidyl transferase–mediated deoxyuridine diphosphate nick-end labelling (TUNEL) test, was observed in the lung, liver and kidney after exposure to the mixture of AFB_1 and FB_1, but also after AFB_1 exposure in the lung (same level as observed in the mixture group) and after FB_1 exposure in the kidney (lower level compared with the mixture). There was an inversion of the typical sphinganine to sphingosine ratio in rats fed with fumonisin alone and combined with AFB_1. The ratio was higher in the kidney of the mixture

group compared with the fumonisin-only group. The Committee considered that the data indicate additivity of the effects of fumonisins and AFB_1 (Theumer et al., 2008).

The cancer initiation potency of FB_1 and AFB_1 was studied in Fischer rats (weaning phase). The rats received a single initiating dose of diethylnitrosamine (200 mg/kg bw; intraperitoneal) or a daily gavage dose (17 µg/kg bw per day) of AFB_1 for 14 days or a diet containing FB_1 at 250 mg/kg (equivalent to 25 mg/kg bw per day) over a period of 21 days (FB_1 from culture, 90–95% purity, 3–4% impurity of monomethyl ester of FB_1). Promotion commenced 3 weeks after initiation by a gavage dose of 2-AAF (20 mg/kg bw per day) on 3 consecutive days followed by partial hepatectomy on day 4. In the second part of the study, sequential exposure was studied by treatment with AFB_1 (17 µg/kg bw per day) by gavage over a period of 14 days followed by FB_1 treatment (250 mg/kg diet) 3 weeks later over a period of 21 days. Adequate control groups were included. Rats were killed 3 weeks after the 2-AAF/partial hepatectomy regimen (initiation potency) and immediately after FB_1 treatment (sequential exposure).

The compounds exhibited slow cancer initiating potency (with and without promotion by 2-AAF) as monitored by the induction of foci and nodules stained positively for the placental form of glutathione *S*-transferase. However, sequential treatment with AFB_1 and FB_1 increased the number and size of $GSTP^+$ lesions significantly compared with the separate treatments. Relative liver weight was reduced by single and sequential AFB_1 and FB_1 exposure. Histology indicated that the individual treatments caused less liver toxicity than sequential treatment. The Committee considered that the data indicate synergistic effects on cancer initiation. However, it cannot be concluded whether a promoting or initiating action of FB_1 together with AFB_1 caused the increase in foci and nodules (Gelderblom et al., 2002a).

Rabbits

Male New Zealand rabbits 50 days of age (six per group) were treated for 21 days by gavage with A) 0 mg FB_1 + 0 µg AFB_1 per kilogram body weight per day (control); B) 0 mg FB_1 + 30 µg AFB_1 per kilogram body weight per day; C) 1.5 mg FB_1 per kilogram body weight per day + 30 µg AFB_1 per kilogram body weight per day; or D) 1.5 mg FB_1 per kilogram body weight per day + 0 µg AFB_1. FB_1 purity was greater than 95%, and AFB_1 was obtained from fungal cultures. Clinical evaluation was performed every 8 hours, and body weight was recorded at the start and end of the experiment. Blood samples were collected at the start and end of the experiment for biochemical analysis. Sphingosine and sphinganine levels were determined in liver, serum and urine. Animals were killed on day 22, gross examination was performed and liver and kidneys were weighed and examined histologically.

Animals from group B (AFB_1) and principally from group C (combined) showed clinical signs of intoxication (apathy, anorexia, lethargy, ruffled fur) and a lower body weight gain. Three animals from group C died. Absolute liver weight was reduced in all treatment groups. Absolute kidney weight was increased in groups B and C. Biochemical parameters indicated impaired hepatic function in

group B and impaired hepatic and renal function in group C (more marked than in group B). Slight indications for hepatic impairment were observed in group D. Disruption of sphingolipid metabolism was observed in all treatment groups, with the largest effect in the liver in group D and in serum and urine in group C. Group D showed congestion of the liver and kidney and vacuolar degeneration of the liver. Group B showed similar results supplemented with enlargement of the gall bladder, disruption of the hepatic structure and hyperplasia of biliary ducts. Most changes were observed in group C, which showed necrotic areas, sinusoidal leukocytosis and lymphocyte depletion in the spleen, in addition to the lesions mentioned for groups B and D. The Committee concluded that a synergistic effect on body weight gain was observed; however, histological lesions and effects on biochemical parameters might indicate an additive effect (Orsi et al., 2007).

Pigs

Weaned piglets (7 weeks old, two neutered males and two females per group) were fed A) control diet or diets containing B) 10 mg of fumonisin B per kilogram of feed (containing FB_1 at 8 mg/kg and FB_2 at 3 mg/kg, equivalent to 0.4 mg of fumonisin B per kilogram body weight per day); C) 30 mg of fumonisin B per kilogram of feed (containing FB_1 at 26 mg/kg and FB_2 at 8 mg/kg, equivalent to 1.2 mg fumonisin B per kilogram body weight per day); D) 50 µg of AFB_1 per kilogram of feed (2 µg/kg bw per day); E) 10 mg of fumonisin B + 50 µg of AFB_1 per kilogram of feed; or F) 30 mg of fumonisin B + 50 µg of AFB_1 per kilogram of feed.

In all piglets from groups C and F, pulmonary oedema was observed. One female piglet from group C died. Feed consumption and body weight gain were decreased in groups C and F. FB_1 was detected in faeces and urine at 24 hours and in liver at 28 days. In groups C and F, erythrocyte number, haematocrit, and total bilirubin, total protein, alkaline phosphatase, AST, ALT and cholesterol levels were increased. Albumin concentrations were increased in groups B, C, E and F. Leukocyte numbers, mean corpuscular haemoglobin and mean corpuscular volume were not affected. The organ to body weight ratios (heart, liver and lung) were greater in groups C and F. In groups C and F, mild hepatic lesions were found. The only joint effects of FB_1 and AFB_1 detected (group F) were decreases in feed consumption and feed conversion. The Committee considered that the data indicate an additive effect of fumonisin B and AFB_1 (Dilkin et al., 2003).

Chickens

Male broiler chicks (12 per group) were fed from day 8 to day 41 of age with diets containing AFB_1 at 0, 0.05 or 0.2 mg/kg (equivalent to 0, 6.25 and 25 µg/kg bw per day, respectively) and FB_1 at 0, 50 or 200 mg/kg (equivalent to 0, 6.25 and 25 mg/kg bw per day, respectively), in a 3 × 3 factorial arrangement. AFB_1 and FB_1 were extracted from fungal cultures (purities unknown). The FB_1 level in the basal diet was 2.23 mg/kg. Animals were vaccinated against Newcastle disease on day 14 of life, and blood was collected at days 35 and 41 to determine humoral antibody titres.

All mycotoxin-treated groups had reduced body weight gain. Changes were more marked in the combination treatments. All mycotoxin-treated groups

had increased relative heart weights. Relative liver weight was increased by 200 mg/kg FB_1 alone or in combination with AFB_1. FB_1-only treatment induced vacuolar degeneration in the liver and cellular proliferation in bile ducts. AFB_1-only treatment at 0.05 mg/kg showed the same, including a change in disposition of cells in the bile duct and infiltrates with inflammatory cells. Treatment with 0.2 mg/kg AFB_1 alone gave more severe lesions, including retraction and disorganization of hepatocytes, focal necrosis and inflammatory cell infiltrates. Combined treatment of AFB_1 + FB_1 resulted in proliferation of bile ducts and infiltrates and furthermore in megalocytosis, heterophilic infiltrates and hydropic degeneration in kidneys. Groups receiving the mycotoxins generally had reduced antibody titres, with birds fed combinations of AFB_1 and FB_1 having the lowest values. The Committee considered that the data indicate that AFB_1 and FB_1 have primarily additive effects on body weight, liver structure and immune response of broilers (Tessari et al., 2006).

Male broiler chicks were fed from days 8 to 41 of age with diets containing AFB_1 at 0, 0.05 or 0.2 mg/kg (equivalent to 0, 6.25 and 25 µg/kg bw per day, respectively) and FB_1 at 0, 50 or 200 mg/kg (equivalent to 0, 6.25 and 25 mg/kg bw per day, respectively), in a 3 × 3 factorial arrangement with nine treatments and 12 birds per treatment. AFB_1 and FB_1 were extracted from fungal cultures (purities unknown).

At 33 days post-feeding, AST levels were higher in all treatment groups with the exception of birds fed FB_1 at 50 mg/kg only. The increase was highest in groups fed AFB_1 at 0.2 mg/kg and in groups fed AFB_1 at 0.05 mg/kg combined with FB_1. Broilers receiving the highest levels of AFB_1 and FB_1 had bile duct proliferation and trabecular disorder in liver samples. AFB_1 alone or in combination with FB_1 at 50 mg/kg caused vacuolar degeneration of the liver and mild cell proliferation in bile ducts. Other lesions are not mentioned. The Committee considered that the data indicate that AFB_1 and FB_1 may have an additive effect (Tessari et al., 2010).

Quail

Japanese quail (48 birds per group) were fed the following diets for 140 days (five 28-day periods): 1) 0 (control); 2) FB_1 at 10 mg/kg (equal to 1.45–1.68 mg/kg bw per day); 3) AFB_1 at 0.05 mg/kg (equal to 7–8 µg/kg bw per day); 4) AFB_1 at 0.05 mg/kg + FB_1 at 10 mg/kg; 5) AFB_1 at 0.2 mg/kg (equal to 29–34 µg/kg bw per day); and 6) AFB_1 at 0.2 mg/kg + FB_1 at 10 mg/kg. AFB_1 and FB_1 were produced from fungal cultures. FB_1 was detected in the basal diet at 0.81 mg/kg. The basal diet was free of AFB, OTA, DON and ZEA.

Feed intake and body weight were lower in birds fed AFB_1 or FB_1, either alone or combined. Egg production was decreased in birds fed FB_1 at 10 mg/kg or FB_1 at 10 mg/kg + AFB_1 at 0.05 mg/kg. Average egg weight was lower in all groups with exposure to AFB_1 or FB_1, either alone or combined. The Committee considered that the data indicate that combined administration of AFB_1 and FB_1 results in additive effects on feed consumption, body weight, egg production and egg weight (Ogido et al., 2004).

In vitro

Rat primary hepatocyte cultures were incubated with AFB_1 (9 nmol/l) and FB_1 (purity 95%, 10, 100 or 250 µmol/l), either alone or combined. The toxins did not decrease cell viability (assessed by flow cytometry after propidium iodine intercalation), but did induce apoptosis (assessed by agarose gel electrophoresis and acridine orange and ethidium bromide staining) in a concentration- and time-dependent manner. The Committee considered that the data suggest that the effects of AFB_1 and FB_1 are additive (Ribeiro et al., 2010).

(iii) Fumonisin and OTA

Rats

In a study in rats, the effects of OTA and FB_1 on oxidative stress in kidney and liver were studied. Rats were treated orally with OTA (5 ng/kg bw and 50 µg/kg bw) and FB_1 (200 ng/kg bw and 50 µg/kg bw) or their combinations. The combination of the low doses of OTA and FB_1 increased the malondialdehyde and protein carbonyl concentrations in the liver and the kidney, compared with controls and animals treated with the individual mycotoxins at the same doses (Domijan et al., 2007b). The Committee noted that effects were observed at very low doses. However, often the effects appeared to lack dose dependency, even if very high doses were used.

The effect of treatment with OTA (5 ng/kg bw) and/or FB_1 (0.05 and 0.5 mg/kg bw) for 5 days on DNA damage in the kidney of rats was tested with the comet assay and the Fpg-modified comet assay. OTA and FB_1 increased DNA damage at all doses. The effects of combined treatment appeared to be additive or less than additive, whereas some data suggested that synergism may occur. The Committee noted that the effects were not always dose dependent (Domijan et al., 2006).

The effect of OTA, FB_1 and their combinations on DNA damage was studied using the standard alkaline comet assay and the Fpg-modified comet assay. Rats received oral doses of OTA (0.005, 0.05 or 0.5 mg/kg bw) for 15 days, FB_1 (0.002, 0.05 or 0.5 mg/kg bw) for 5 days and combinations of the two lower OTA and FB_1 doses (animals were treated with OTA for 15 days and with FB_1 for the last 5 days of OTA treatment). In OTA- or FB_1-treated animals, the tail length, tail intensity and Olive tail moment in the standard comet assay and Fpg-modified comet assay were higher than in controls, at all doses tested. The Fpg-modified comet assay showed greater tail length, tail intensity and Olive tail moment than did the standard comet assay, suggesting that oxidative stress is responsible for the DNA damage. DNA damage detected by the standard comet assay at all OTA or FB_1 doses indicates that another mechanism is also involved. The Committee considered that the combined OTA + FB_1 treatment showed a synergistic effect in the standard and modified comet assays (Domijan et al., 2006).

In vitro

The combined effects of OTA and fumonisin were studied in vitro by measuring neutral red uptake in three cell lines: C6 glioma cells, Caco-2 cells and Vero cells. The data suggest synergistic cytotoxic effects of these mycotoxins as

measured by increased uptake of neutral red in the three cell lines (Creppy et al., 2004).

Human and porcine lymphocytes were incubated with 5 or 20 µg/ml of FB_1 or OTA or the combination of the two mycotoxins for up to 96 hours. Cell viability was assessed by the MTT assay. A progressive decrease in cell viability was observed with increasing time of exposure to the mycotoxins. FB_1 had a lower cytotoxic effect than OTA. The Committee considered that the data suggest a synergistic activity of these mycotoxins (Mwanza et al., 2009).

(iv) Fumonisin and moniliformin

Pigs

Four groups of six barrows (three replicates of two each; mean body weight, 11.1 kg) were fed diets containing control feed, 100 mg of FB_1 per kilogram feed, 100 mg of MON per kilogram of feed or 100 mg of FB_1 + 100 mg of MON per kilogram of feed for 28 days. Body weight gain, feed efficiency, serum biochemical analytes and haematological values were adversely affected by the FB_1 and the FB_1 + MON diets. Two barrows in the MON group and two barrows in the FB_1 + MON group died during the first 6 days of the study. Mild to moderate lesions were observed microscopically in heart and lung tissues of the groups fed MON and FB_1 + MON and in liver tissues of the groups fed FB_1 and FB_1 + MON. The Committee considered that the effects induced by the combined feeding appear to be additive or less than additive (Harvey et al., 2002).

Chickens

Chicks were fed FB_1 (0, 100 or 200 mg/kg diet), MON (0, 100 or 200 mg/kg diet) or mixtures of both mycotoxins, using a 3 × 3 factorial design. Mortality (50–65%) was induced by MON at 200 mg/kg diet. Feed intake and body weight gain were decreased in chicks fed diets containing MON at 100 mg/kg. Chicks fed MON had increased heart weights. Increased kidney and liver weights were induced by MON at 200 mg/kg diet or by a combination of FB_1 at 200 mg/kg diet and MON at 100 mg/kg diet. FB_1 induced periportal extramedullary haematopoiesis and mild focal hepatic necrosis. The incidences of large pleomorphic cardiomyocyte nuclei, loss of cardiomyocytes and mild focal renal tubular mineralization were increased by MON. Both cardiac and renal lesions were induced by mixtures of FB_1 and MON. The Committee concluded that the effects of FB_1 and MON were less than additive (Ledoux et al., 2003).

Quail

In studies in young quail, it was found that the continuous presence of *Fusarium moniliforme* culture material in the diets might increase their susceptibility to or the severity of *Salmonella gallinarum* infection (Deshmukh et al., 2005, 2007).

Japanese quail received diets containing FB_1 (200 mg/kg, from *Fusarium verticillioides* culture material) and/or MON (100 mg/kg, from *Fusarium fujikuroi*

culture material). Except for mortality (additive effect) and serum AST levels (less than an additive effect), no interactions between FB_1 and MON were observed for the other variables measured (Sharma et al., 2008).

(v) Fumonisin and deoxynivalenol

In a 5-week study, piglets (5 weeks old, six per group) received control diet or diets containing DON (3 mg/kg) or fumonisin B (6 mg/kg) or both toxins. At days 4 and 16, the animals were immunized with ovalbumin to assess their specific immune response. The diets had minimal effects on haematological and biochemical blood parameters. DON and fumonisin B induced histological lesions in the liver, lungs and kidneys. The liver was more affected by a mixture of DON and fumonisin B. Anti-ovalbumin IgG levels in plasma and lymphocyte proliferation upon antigenic stimulation were reduced. The expression of IL-8, IL-1β, IL-6 and macrophage inflammatory protein-1β was decreased in the spleen (Grenier et al., 2011).

(vi) Fumonisin and zearalenol

Treatment of human lymphoblastoid Jurkat T cells with FB_1 (20–150 µmol/l) resulted in a dose-dependent induction of proliferation. α-ZEA (10–80 µmol/l) showed a marked inhibitory effect on cell proliferation, essentially mediated by apoptosis. In stimulated cells pre-incubated with FB_1, the levels of IL-2 and IFN-γ mRNAs were similar to control, whereas a reduction of cytokine transcripts was reported following α-ZEA treatment. Mixtures of FB_1 and α-ZEA resulted in further inhibition of both proliferation and IFN-γ mRNA expression (Luongo et al., 2006).

(vii) Fumonisin and multiple mycotoxins

Caco-2 cells were incubated for 72 hours with FB_1 (10 µmol/l), DON (4, 10 or 20 µmol/l), ZEA (5, 10 or 20 µmol/l) or combinations of two or three treatments. Malondialdehyde production (lipid peroxidation), inhibition of protein synthesis and DNA synthesis, DNA methylation (percentage of 5-methylcytosine in DNA), DNA fragmentation and cell viability were assessed.

The mixtures of mycotoxins reduced cellular viability (as measured by the neutral red test) in increasing order: [FB_1 + ZEA] < [FB_1 + DON] < [ZEA + DON] < [FB_1 + DON + ZEA]. Because FB_1 antagonizes the effects of estrogenic ZEA, FB_1 was assayed against estradiol. A mixture of FB_1 and estradiol and/or ZEA improved Caco-2 cell viability in contrast to individual effects. Lipid peroxidation was increased by the mycotoxins, with mixtures of ZEA or FB_1 and DON displaying additive effects. Tertiary exposure did not further increase lipid peroxidation. DNA synthesis was inhibited by ZEA, DON and FB_1, with the effects of binary and tertiary mixtures being far less than additive. Each toxin inhibited protein synthesis, with mixtures showing an additive effect. ZEA, DON and FB_1, alone or in combination, induced DNA fragmentation. Each individual mycotoxin (10 µmol/l) raised the percentage of 5-methylcytosine in DNA from 4.5% to 9%. The combinations did not increase this rate any further. The data indicate that mixtures of *Fusarium* toxins induce lipid peroxidation, DNA damage, DNA fragmentation, DNA methylation and cytotoxicity

in Caco-2 cells and suggest a potential promoter effect in human intestinal cells. The binary mixtures appear to have an additive effect based on cell viability, lipid peroxidation and protein synthesis. Most tertiary treatments did not add to the effects, compared with binary treatments. The additive effect was not observed for DNA synthesis, DNA methylation and, in the case of FB_1 and ZEA, viability (Kouadio et al., 2007).

Human peripheral blood mononuclear cells were incubated with FB_1, OTA, PA and CIT or mixtures of these mycotoxins. A mixture of OTA (12.5 mg/l), CIT (125 mg/l) and FB_1 (125 mg/l) induced a stronger suppression of metabolic activity of PHA-stimulated peripheral blood mononuclear cells (31.2%) than OTA (38%), CIT (42%) or FB_1 (55.6%) alone. Inhibition of cell proliferation was stronger for the mixture (33%) than for OTA (43%), CIT (44%) and FB_1 (56%) alone (Stoev et al., 2009).

Porcine kidney epithelial cells were incubated for 24 or 48 hours with 0.05, 0.5 or 5 µg/ml of FB_1, OTA or BEA or combinations of two or all three mycotoxins. After 24 hours, cell viability was significantly decreased by 5 µg/ml of FB_1 (25%), BEA (30%) and OTA (35%). At 5 µg/ml, OTA increased lipid peroxidation at 24 hours (56%) and 48 hours (85%). Increased lipid peroxidation was induced by FB_1 (57%) and BEA (80%) at 5 µg/ml only at 48 hours. At 24 hours, glutathione concentrations were significantly decreased by OTA (18%) at 0.05 µg/ml and by FB_1 (11%) and BEA (13%) at 0.5 µg/ml. The Committee considered that the effects of combined treatment with two or three of the mycotoxins appeared to be additive (Segvic Klaric et al., 2006).

Porcine kidney epithelial PK15 cells were treated in vitro for 24 or 48 hours with 0.05, 0.5 or 5 µg/ml of FB_1, OTA and BEA or the combination of two or all three mycotoxins. At 48 hours, significant increases in LDH activity were induced by 5 µg/ml of FB_1 (45%), BEA (84%) and OTA (77%). OTA at 0.5 µg/ml increased caspase-3 activity at 24 hours (84%), whereas at the same dose, increases were observed at 48 hours with BEA (319%) and FB_1 (419%). Apoptosis was observed after 48 hours of incubation with a single toxin. The Committee concluded that, generally, the effects of combined exposure appeared to be additive (Segvic Klaric, Pepeljnjak & Ruzica, 2008).

Swine whole blood cultures were incubated with four *Fusarium* toxins: FB_1, α-ZEA, NIV and DON. Increasing concentrations of FB_1 (0.5–80 µmol/l) did not affect cell proliferation. α-ZEA (0.5–20 µmol/l), NIV (0.0625–2 µmol/l) and DON (0.0625–2 µmol/l) induced an inhibitory effect. A combination of FB_1 + α-ZEA produced synergistic inhibition of porcine cell proliferation, whereas no interaction between DON and NIV was observed (Luongo et al., 2008).

The effect of aflatoxin B and fumonisin B on morphology, capacity of cellular proliferation, cytotoxicity and IL-8 synthesis in a porcine intestinal epithelial cell line (iPEC-1) was studied. Cellular morphology was affected only at concentrations higher than 50 µmol/l for aflatoxin B and 500 µmol/l for fumonisin B. Cellular proliferation, cellular damage and synthesis of IL-8 were affected by aflatoxin B/fumonisin B mixtures of 1.3/3.7, 2/3.7 and 5/10 µmol/l. The Committee considered

that the data indicate that the combination of aflatoxin B and fumonisin B may have synergistic effects (del Rio Garcia et al., 2007).

PK15 cells were incubated for 24 and 48 hours with 0.05, 0.5 and 5 mg/ml of FB_1, BEA and OTA or combinations of two or all three mycotoxins in equal concentrations. Giemsa-stained binucleated PK15 cells were scored for the presence of micronuclei, nuclear buds and nucleoplasmic bridges. Single mycotoxins induced micronuclei, nuclear buds and nucleoplasmic bridges in a dose-dependent manner, showing predominantly a clastogenic effect. OTA showed stronger genotoxic potential than FB_1 and BEA. Mycotoxin combinations increased the frequency of micronuclei and nucleoplasmic bridges, mostly in an additive manner (Segvic Klaric et al., 2008).

(vii) Fumonisin and other agents

Rats

In a 4-week study, female Sprague-Dawley rats (10 per group) received A) control diet or B) diet containing fumonisin B at 200 mg/kg (equivalent to 10 mg/kg bw per day). Groups C and D received oral treatment with ethanol extract of *Aquilegia vulgaris* at 5 and 10 mg/kg bw, respectively. Groups E and F were fed the fumonisin B–contaminated diet and treated with the ethanol extract at 5 and 10 mg/kg bw, respectively. Fumonisin B (68% FB_1, 21% FB_2 and 11% FB_3) was produced from fungal cultures.

No mortality was observed. Treatment with fumonisin B increased total cholesterol, triglycerides, low-density lipoprotein and sphinganine to sphingosine ratio and decreased high-density lipoprotein. Furthermore, in the fumonisin B group, glutathione and antioxidant capacity were decreased, lipid peroxidation was increased, micronucleated polychromatic erythrocytes in bone marrow were increased, alterations in DNA were observed (random amplification of polymorphic DNA PCR) and DNA and RNA levels in the liver were increased, accompanied by necrosis, inflammatory cells and fibrous tissue. The *A. vulgaris* extracts did not induce any significant changes in the biochemical or histological picture. The combined treatments showed significant improvements in all biochemical and cytogenetic parameters tested and histological pictures in the liver tissues, which were more pronounced in the group that received the high dose of the extract (Hassan et al., 2010).

Female Fischer rats (five rats per group) received A) standard diet, B) diet with 70% of *Ustilago maydis* galls (equivalent to 35 g/kg bw per day) or C) diet containing 70% of *U. maydis* galls and 1 mg/kg FB_1 (equivalent to 0.05 mg/kg bw per day) for 17 days. FB_1 was obtained from fungal cultures (purities unknown).

Animals from groups B and C were more excited, showing hyperactivity, increased feed consumption and slightly increased body weight gains. Hyperaemia, swollen tissue and fragile tissue were observed in liver, kidneys, brain, lungs, intestine, uterus and ovaries, but not in heart or spleen. Acetylcholinesterase and GGT activities were decreased in the liver and brain of group B. Acetylcholinesterase and alkaline phosphatase activities were decreased in the liver of group C. In group

C, LDH in the blood serum was increased, whereas ALT was decreased, indicating that the LDH increase was not caused by liver injury. The data suggest that *U. maydis* and FB_1 showed neurotoxicity in Fischer rats, which could be related to the alkaloids of *U. maydis* and disruption of sphingolipid metabolism by FB_1 activity. The Committee noted that no conclusions can be drawn about the interaction of *U. maydis* and FB_1, as no exposure with FB_1 alone was included (Pepeljnjak, Petrik & Segvic Klaric, 2005).

Pigs

Male and female piglets (34 days of age) were distributed into five groups (five piglets per group, number of males and females per group unknown): A) negative control, B) orally treated using a probe with 12 mg/kg bw per day of FB_1 (purity 98%) starting from day 0; C) inoculated with porcine reproductive and respiratory syndrome virus (PRRSV) on day 8; D) inoculated with PRRSV and 12 mg/kg of FB_1 starting from day 0; or E) inoculated with 12 mg/kg of FB_1 starting from day 0 and inoculated with PRRSV on day 8.

A negative slope effect was observed in weight gain. Groups D and E showed weight loss with the presence of PRRSV and FB_1. Weight gain in groups B and C was reduced compared with the control. All treated piglets showed anorexia and depression. Hyperaemia was observed in groups C, D and E from day 14 onward. FB_1 treatment also resulted in fluid stools and prostration, and PRRSV injection also caused swollen eyelids, fluid at nostrils, dyspnoea and rough hair. In groups D and E, anorexia, depression, fluid at nostrils, sneezing and fluid stools were observed. Macroscopic examination of the lungs showed reddish consolidation in groups B, D and E and diffuse purple consolidation in groups C, D and E. Histological examination of these lungs revealed thickened alveolar septa with mononuclear infiltrate, with vascular changes and clean alveoli characteristic of interstitial pneumonia. In group D, moderate diffuse interstitial pneumonia was observed. Group E showed severe interstitial lung lesion. Polyploidy and binuclear cells were observed in the liver, suggesting mycotoxicosis in groups B, D and E. In groups B, D and E, proliferative glomerulonephritis and atrophy of glomeruli were observed. The Committee considered that the data indicate that PRRSV and FB_1 have an additive effect in swine (Moreno Ramos et al., 2010).

Piglets (sex and age unknown) received by gavage vehicle or a crude extract of fumonisin (containing 54% FB_1, 8% FB_2 and 9% FB_3). The dose administered was 0.5 mg FB_1 per kilogram body weight per day for 7 days. At day 8, half of the animals were instilled intratracheally with a non-toxin-producing type A strain of *Pasteurella multocida*. Piglets treated with both fumonisin and *P. multocida* had a lower weight gain at days 13–20. Combined treatment also induced coughing during days 9–20 and lung lesions (interstitial pneumonia). Piglets treated with fumonisin B or *P. multocida* showed enlargement of alveolar septa due to an increase in macrophages and lymphocytes. The combined treatment induced exudates consisting of macrophages and lymphocytes in the alveoli and alveolar walls and thickened bronchial parenchyma. Fumonisin B increased the expression of IL-8, IL-18 and IFN-γ mRNA. *Pasteurella multocida* increased the expression of TNFα; fumonisin B and *P. multocida* combined induced increased mRNA expression of

TNFα, IFN-γ and IL-18. It is not clear if the outcome in the combined treatment group is based on an additive or synergistic effect (Halloy et al., 2005).

2.3 Observations in domestic animals and veterinary toxicology

Consumption of feeds contaminated with fumonisin is a proven cause of two farm animal diseases and a suspected cause of others (Annex 1, reference *153*; IPCS, 2000). In horses, the neurotoxic disease known as ELEM is a fatal disease that occurs only in equids (horses and related species). The disease is characterized by the presence of liquefactive necrotic lesions in the white matter of the cerebrum; the grey matter may also be involved (IPCS, 2000).

The other farm animal disease proven to be caused by fumonisin is porcine pulmonary oedema (PPO) syndrome (Annex 1, reference *153*; IPCS, 2000). PPO is a rapid-onset and often fatal disease that is usually associated with consumption of feeds heavily contaminated with fumonisins. Like ELEM, signs of liver toxicity are common in animals exhibiting signs of PPO.

In recent years, reports of ELEM and PPO have been rare. Much of the recent published research on ELEM and PPO has been focused on the biochemical and physiological basis for the diseases, which will be summarized briefly, as the physiological mechanisms have relevance to humans. Both ELEM and PPO are believed to result from cardiovascular deregulation resulting from fumonisin-induced disruption of sphingolipid metabolism (Smith et al., 2002; Constable et al., 2003; Hsaio et al., 2005). For ELEM, the downstream effects associated with sphingolipid-induced alterations in cardiovascular function include deregulation of cerebral arteries responsible for autoregulation of blood flow to the horse's brain (Haschek, Voss & Beasley, 2002; Smith et al., 2002; Foreman et al., 2004). PPO is hypothesized to be a result of acute left-sided heart failure as a consequence of sphingoid base–induced inhibition of L-type calcium channels (Haschek, Voss & Beasley, 2002). For both diseases, it is likely that fumonisin exposure results in disruption of signalling pathways associated with the extracellular G-protein coupled sphingosine 1-phosphate receptors, $S1PR_{1-5}$ (formerly endothelial differentiation gene receptors) (see Figure 2 above). Typically, the $S1PR_1$ (and possibly other sphingosine 1-phosphate receptors) functions to maintain endothelial barrier homeostasis (reviewed in Gelineau-Van Waes et al., 2009; Maceyka, Milstien & Spiegel, 2009). Fumonisin B–induced deregulation of sphinganine 1-phosphate production and the increased level of sphinganine 1-phosphate in blood will likely disrupt normal $S1PR_1$ regulation of vascular permeability and other aspects of vascular physiology (reviewed in Gelineau-Van Waes et al., 2009).

In both horses and pigs, the elevation in free sphingoid bases in serum, liver, kidney (Annex 1, reference *153*; IPCS, 2000) and other tissues (Tumbleson et al., 2003) and serum sphinganine 1-phosphate levels (Constable et al., 2005; Piva et al., 2005) are biomarkers for exposure to potentially toxic levels of fumonisins. These biomarkers can be used in the field along with feed analysis and behavioural changes for diagnostic purposes (Riley et al., 2011). The earliest neurological sign of fumonisin toxicosis in horses is loss of tongue tone and movement, which precedes all other neurological signs (Foreman et al., 2004). This may have been described in earlier natural outbreaks of ELEM as dysphagia (Foreman et al., 2004).

An observational approach for early detection and possible treatments to minimize losses during an outbreak of ELEM has been described (Foreman et al., 2004). The NOAEL (intravenous) for the cardiovascular changes believed to be the cause of ELEM is 0.01 mg/kg bw per day, which is equal to 8 mg/kg diet (Foreman et al., 2004; see section 2.2.2 for the summary of the toxicological aspects of the study).

Although ELEM and PPO are clearly fumonisin-induced diseases in farm animals, fumonisin-induced performance problems are believed to be a subtle cause of economic loss to farmers through reduced weight gain, decreased reproductive efficiency, decreased egg and milk production and increased susceptibility to stress and infectious disease (CAST, 2002). Exposure in farm animals is potentially much higher than in humans, as maize is a major ingredient in animal feeds.

The physiological basis for performance problems in pigs, poultry and other farm animals induced by fumonisin is unclear; however, several in vitro and in vivo studies since 2001 have shown that fumonisin exposure can have deleterious effects on intestinal integrity and function, which can lead to altered intestinal immune responses and possibly other effects on intestinal physiology (Bouhet et al., 2004, 2006; Loiseau et al., 2007; Devriendt et al., 2009; Lessard et al., 2009). None of these studies was designed to establish a NOAEL, but they do reveal potential risks to both farm animal productivity and health and possible contributing factors to human risk.

Oral exposure to fumonisin also has effects on other immune responses, including sex-specific decreased antibody titres after vaccination and increased susceptibility to secondary pathogens in pigs (Oswald et al., 2003, 2004; Halloy et al., 2005; Marin et al., 2006) and poultry (Deshmukh et al., 2007), all of which could have effects on farm animal performance. None of these studies were designed to establish a NOAEL, but the study by Marin et al. (2006) did show a NOAEL in female pigs for immunotoxicity. This study has been described in section 2.2.6(a).

It has been suggested that mycotic nephropathy in pigs and chickens is a result of concurrent exposure to multiple mycotoxins, including fumonisin, OTA and PA (Stoev et al., 2010). This is not unreasonable, as fumonisins cause liver damage in all farm animals tested and also kidney damage in rabbits, cattle and sheep or the equivalent organs in fish.

The relative sensitivity of farm animals to fumonisin toxicity, as reflected in the United States Food and Drug Administration's (USFDA) guidance to industry for total fumonisins in animal feed, is equids and rabbits > pigs and catfish > breeding ruminants, breeding poultry and breeding mink > ruminants 3 months or older being raised for slaughter and mink being raised for pelt production > poultry being raised for slaughter (USFDA, 2001).

Since the last JECFA evaluation (Annex 1, reference *153*), there have been several studies evaluating various aspects of fumonisin toxicity in pigs, horses and other species, which are described in section 2.2.2.

2.4 Observations in humans

This section on epidemiological studies of fumonisin exposure and health effects includes two of the same health end-points as the previous fumonisin

evaluation (Annex 1, reference *153*): oesophageal cancer and NTDs in babies. Two new health conditions that have been associated with fumonisin since the 2001 evaluation are also included in this section: 1) human immunodeficiency virus (HIV) and 2) childhood stunting. One health condition in the 2001 evaluation that is not in this report is acute toxicosis caused by fumonisins, as there have been no documented cases of acute fumonisin toxicity in humans since the ones recorded in the 2001 evaluation. At the end of this section, section 2.4.5 describes recent work on identifying and validating human biomarkers of fumonisin exposure.

The studies described are those that have been published in the public literature since 2001 and hence were not included in the previous evaluation by the Committee. This document does not include discussions of studies that measured fumonisin exposure but did not link this exposure with either biomarkers or any specific human health effects. Nor does it describe studies done on human cell culture assays in vitro.

Table 9 summarizes the epidemiological studies conducted since the previous evaluation by the Committee (Annex 1, reference *153*) that assess the relationship between fumonisin exposure and a variety of adverse health effects. For each category of health effect, the geographic region, the reference, the age-standardized incidence of the health effect and the levels of fumonisin or potential fumonisin biomarkers or other mycotoxins are listed.

2.4.1 Oesophageal cancer

There are about 481 000 documented cases of oesophageal cancer worldwide each year (IARC, 2008). Two important types of oesophageal cancer are squamous cell carcinoma and adenocarcinoma. The former is the predominant form of global oesophageal cancers, making up 90–95% of all cases worldwide. The latter is more common in industrial nations and is associated with gastro-oesophageal reflux disease, which may lead to Barrett oesophagus. Oesophageal squamous cell cancer has been associated with smoking and alcohol consumption, primarily in males, and it is suspected that fumonisin exposure may be an additional risk factor for this cancer.

The report of the previous evaluation by the Committee (Annex 1, reference *153*) describes studies linking oesophageal cancer to fumonisin exposure in multiple nations worldwide. In the last decade, the epidemiological studies that have drawn associations between oesophageal cancer incidence and fumonisin exposure were conducted in China, South Africa and the Islamic Republic of Iran.

Sun et al. (2007) collected maize samples from three Chinese counties: Huantai (low incidence of both oesophageal and liver cancers), Huaian (high incidence of oesophageal cancer: >80 cases per 10 000 population) and Fusui (high incidence of liver cancer: >50 cases per 10 000 population). FB_1 was detected in high proportions of the samples from all three counties: 95.7% from Huaian, 83.0% from Fusui and 83.3% from Huantai. However, the average concentrations of FB_1 among the positive samples from each county varied significantly: 2.84 mg/kg in Huaian, 1.27 mg/kg in Fusui and 0.65 mg/kg in Huantai. The authors stated that these results suggest that FB_1 may contribute to oesophageal cancer

Table 9. Summary information on epidemiological studies associating fumonisin exposure with health effects—oesophageal cancer, human immunodeficiency virus, stunting and neural tube defects

Geographic region/country	Reference	Age-standardized incidence (cases per 100 000)	Mycotoxin contamination or biomarker levels
Oesophageal cancer			
China, Huaian, Fusui and Huantai counties	Sun et al. (2007)	Huaian >800; Huantai <100	FB_1: Fusui 1.27 (0.1–14.9) mg/kg; Huaian 2.84 (0.1–25.5) mg/kg; Huantai 0.65 (0.1–5.7) mg/kg
China, Huaian, Fusui and Huantai counties	Sun et al. (2011)	Huaian: high risk, oesophageal cancer; Fusui: high risk, liver cancer; Huantai: low risk, oesophageal and liver cancer	Huaian maize: AFB_1 13.5 mg/kg, FB_1 2.6 mg/kg; Fusui maize: AFB_1 2.3 mg/kg, FB_1 0.4 mg/kg, plant oil 52.3 mg/kg; Huantai maize: AFB_1 1.3 mg/kg, FB_1 0.3 mg/kg
South Africa, Bizana and Centane	Shephard et al. (2007)	N/A	FB_1: 281 ± 262 (38–1066) ng/ml; total fumonisins: 369 ± 345 (43–1329) ng/ml
Islamic Republic of Iran, Mazandaran and Isfahan provinces	Shephard et al. (2002a)	N/A	FB_1: Mazandaran 3.18 (0.68–7.66) mg/kg; Isfahan 0.22 (<0.01–0.88) mg/kg
China, Linxian County, Henan Province	Abnet et al. (2001)	150 men, 125 women	No significant associations were found between cancer incidence and the geometric means of any of these potential biomarkers: sphingosine in nmol/l (60.7 [SD 22.2] in cases, 63.3 [SD 26.0] in controls), sphinganine in nmol/l (48.2 [SD 44.3] in cases, 54.6 [SD 52.5] in controls) and the ratio of sphinganine to sphingosine (0.79 [SD 0.75] in cases, 0.86 [SD 0.90] in controls)

Table 9 (contd)

Geographic region/country	Reference	Age-standardized incidence (cases per 100 000)	Mycotoxin contamination or biomarker levels
HIV			
Sub-Saharan Africa	Williams et al. (2010)	435	N/A
Stunting			
United Republic of Tanzania	Kimanya et al. (2010)	Children under age of 5: Stunting: 38% Underweight: 22% Wasting: 3%	Total fumonisins: 0.158 (0.021–3.201) mg/kg; FB_1: 0.106 (0.021–2.375) mg/kg; FB_2: 0.067 (0.020–1.076) mg/kg; FB_3: 0.060 (0.018–0.604) mg/kg
Neural tube defects			
Texas, USA	Missmer et al. (2006)	270	Total fumonisin: 234 (0–1690) ng/g

N/A, not available; SD, standard deviation

risk in Huaian. This study did not control for possible confounders or cofactors contributing to cancer risk, such as socioeconomic status, agroecological zone or other risk factors for oesophageal cancer, such as tobacco smoking and alcohol consumption.

In Sun et al. (2011), two mycotoxins were analysed in maize samples: FB_1 and AFB_1. Based on measurements of FB_1 and AFB_1 in maize samples in Huaian, Fusui and Huantai counties and consumption patterns of the corresponding populations, average daily exposures to both mycotoxins were estimated. Average daily exposure to FB_1 was estimated to be 460 µg in Huaian, 138.6 µg in Fusui and 92.4 µg in Huantai. Average daily exposure to AFB_1 was estimated to be 1.723 µg in Huaian, 2.685 µg in Fusui and 0.397 µg in Huantai. Again, the trend appeared to show that higher fumonisin exposure is linked with higher oesophageal cancer incidence. The study did not control for possible confounders or cofactors, such as socioeconomic status, agroecological zone or other risk factors for oesophageal cancer.

Similarly, in South Africa, differential fumonisin exposure in communities was linked to differential oesophageal cancer risk. Shephard et al. (2007) estimated average daily dose of fumonisin in adults in two subsistence farming communities—Bizana and Centane—in the former Transkei region of Eastern Cape Province, South Africa, by estimating maize consumption and using previously determined levels of FB_1 and FB_2 in maize from these communities. Adults in Centane, an area with high oesophageal cancer incidence, received an average dose of 8.67 ± 0.18 µg/kg bw per day, whereas adults in Bizana, with relatively low oesophageal cancer incidence, received an average dose of 3.43 ± 0.15 µg/kg bw per day. The authors noted, as well, that mean fumonisin exposures in all age groups in these two communities exceeded the PMTDI of 2 µg/kg bw. Numerical incidence rates for oesophageal cancer in these two communities were not provided in the manuscript.

Finally, FB_1 levels were measured in maize samples from two provinces in the Islamic Republic of Iran in 1999: Mazandaran, with high oesophageal cancer incidence; and Isfahan, with low oesophageal cancer incidence (Shephard et al., 2002a). The 20 maize samples from Mazandaran were gathered randomly from farmers' lots and were found to contain a mean FB_1 level of 3.18 (0.68–7.66) mg/kg. The 10 maize samples from Isfahan were purchased as maize cobs in local retail markets and were found to contain a mean FB_1 level of 0.22 (<0.01–0.88) mg/kg. FB_1 levels were thus significantly different in these two provinces, with the higher levels corresponding to the population at high risk of oesophageal cancer. Numerical incidence rates for oesophageal cancer in these two communities were not provided in the manuscript.

In one study (Abnet et al., 2001), the authors did not measure fumonisin levels directly in foods, but instead measured serum sphingosine, sphinganine and the sphinganine to sphingosine ratio and attempted to correlate these concentrations with oesophageal squamous cell cancer in Linxian County, China. These serum sphingoid bases and their ratio have been proposed in the past as potential biomarkers of fumonisin exposure in humans. Ninety-eight cases and 185 controls participated in the study. After adjusting for age, sex, and tobacco and

alcohol consumption, no significant associations were found between oesophageal cancer incidence and the geometric means of any of these potential biomarkers: sphingosine (in nmol/l) (60.7 [SD 22.2] in cases, 63.3 [SD 26.0] in controls), sphinganine (in nmol/l) (48.2 [SD 44.3] in cases, 54.6 [SD 52.5] in controls) and the sphinganine to sphingosine ratio (0.79 [SD 0.75] in cases, 0.86 [SD 0.90] in controls). However, the Committee considered that this study should not be interpreted to mean that fumonisin is *not* associated with oesophageal cancer, as the sphingoid bases and their ratio have not been validated as human biomarkers of fumonisin exposure.

2.4.2 Human immunodeficiency virus

HIV, discovered in 1981, is a retrovirus that causes the disease acquired immunodeficiency syndrome (AIDS). AIDS is characterized by a progressive deterioration of the acquired, or adaptive, immune system, which increasingly predisposes affected individuals to mortality or morbidity from opportunistic infections or cancers. HIV transmission occurs primarily through unsafe sex, contaminated needles or mother-to-child in utero, at birth or through breastfeeding. It has been estimated that from 1981 to 2006, AIDS killed over 25 million people worldwide. A disproportionate number of these deaths have occurred in Africa. In recent years, however, the number of deaths per year from AIDS has been declining all over the world. In 2009, an estimated 1.8 million people died of AIDS, compared with 2.1 million in 2004. This decrease is at least in part due to increased access to and improvements in HIV antiretroviral therapies (UN, 2010).

One study has attempted to link fumonisin exposure with HIV mortality in sub-Saharan Africa (Williams et al., 2010). In this study, the authors used statistical methods (univariate correlations, linear regression with different combinations of independent variables) to analyse which dietary, cultural and socioeconomic factors appeared to be linked with HIV mortality in 37 sub-Saharan African nations. Variables included national mortalities from oesophageal cancer, liver cancer and hepatitis B virus; proportion of Muslims in the national population; per capita gross domestic product; and average per capita daily consumption of cassava, peanut, maize and rice. It was found that, on a per nation basis, HIV mortality was inversely related to the proportion of Muslims ($R = -0.45$, $P < 0.01$). Oesophageal cancer mortality was directly related to maize consumption ($R = 0.55$, $P < 0.001$) but inversely related to peanut, cassava and rice consumption. Liver cancer mortality was not directly associated with maize consumption, but was directly associated with rice consumption. Nations were labelled “high maize consumption” or “low maize consumption” based on whether they ranked above or below the median of maize consumption among sub-Saharan African nations. The relative risk for “high maize consumption” in HIV mortality was 1.31 (95% confidence interval 1.20–1.42).

Although neither actual fumonisin concentrations in food nor fumonisin exposures in humans were measured, the authors suggested that fumonisin exposure might be related to HIV mortality in Africa. Their train of logic linking fumonisin exposure to HIV mortality is the following: In several studies worldwide, oesophageal cancer has been associated with fumonisin exposure, whereas it is

known that liver cancer is caused by aflatoxin exposure. In the authors' regression analyses among sub-Saharan African nations, maize consumption at a national level was linked to oesophageal but not liver cancer mortality. Maize consumption was also linked to HIV mortality. Thus, it must have been fumonisin in maize, not aflatoxin, that had an influence over HIV mortality. The authors then mentioned two possible mechanisms by which fumonisin exposure might contribute to HIV infection. One is increased membrane permeability, which could promote HIV transmission; the other is disruption of sphingolipid biosynthesis, which could affect how HIV attaches to human cells.

2.4.3 Stunting

Chronic malnutrition is determined by three main indicators for children aged 5 years and under: stunting, underweight and overweight. Stunting, the most commonly used indicator, is defined as a condition in which a child's height for his or her age is 2 standard errors or more below the median value of a WHO reference population; in other words, his height-for-age *z* score is less than or equal to –2 (Ricci et al., 2006; WHOSIS, 2008). It has been estimated that there are about 195 million stunted children in the world, primarily in sub-Saharan Africa, South Asia and South-east Asia (Black et al., 2008). Stunting is a critical condition to control worldwide, because stunted children are more likely to have long-term developmental and cognitive problems and to be susceptible to infectious diseases (Ricci et al., 2006).

One epidemiological study has linked fumonisin exposure with childhood stunting (Kimanya et al., 2010) in the United Republic of Tanzania. Other mycotoxins, such as aflatoxin, may be associated with childhood stunting as well (Gong et al., 2002, 2004); however, the authors noted that aflatoxin exposure was not high in the areas of the United Republic of Tanzania that were relevant to this study. In these areas of the United Republic of Tanzania, weaning and complementary foods for infants and young children are primarily maize based. Fumonisin exposure was estimated for 215 infants aged 6 months or older by measuring fumonisin levels (FB_1 + FB_2 + FB_3) in ready-to-cook maize flour and estimating the infants' daily exposure based on mothers' dietary recall. Percentages of infants in high- and low-exposure groups were compared using chi-squared tests. The associations of fumonisin exposure with height and weight were analysed using a multilevel mixed-effects linear regression model, controlling for the covariates of the child's home village, energy and protein intake from complementary food, and sex.

Of the 215 infants, 191 consumed at least some maize according to their mothers; and of the maize flour samples from these 191 homes, 131 contained detectable fumonisins. For the 131 infants who consumed maize flour with detectable fumonisins, fumonisin exposures ranged from 0.003 to 28.8 µg/kg bw per day (median: 0.48 µg/kg bw per day; 90th percentile: 4.0 µg/kg bw per day). It was found that, when they reached 12 months of age, the 26 infants whose daily total fumonisin exposure was estimated to exceed the PMTDI of 2 µg/kg bw were significantly shorter (1.3 cm) and lighter (328 g) on average than the 105 infants who were exposed to fumonisins but whose daily exposures were not estimated to exceed the PMTDI. Fumonisin exposure was significantly associated with length

and weight of 12-month infants even when other factors were taken into account, such as total energy intake, sex, village and total protein intake.

2.4.4 Neural tube defects

NTDs are embryonic defects of the brain and spinal cord that result from the failure of the neural tube to close in utero (Missmer et al., 2006). The two most common NTDs are spina bifida, in which the fetal spinal column does not close completely during the first month of pregnancy, usually resulting in nerve damage and partial leg paralysis; and anencephaly, in which much of the brain does not develop, leading to stillborn births or death within several hours of birth. Maternal folate consumption, particularly in the first trimester, is critical to reducing the risk of NTDs in fetuses. Because fumonisins disrupt sphingolipid metabolism and hence folate transport across cell membranes (Marasas et al., 2004), they have been shown to induce NTDs in laboratory mice (Gelineau-Van Waes et al., 2005) and therefore may play a role in NTD incidence in high-risk human populations.

One key epidemiological study (Missmer et al., 2006) showed an association between estimated fumonisin exposure during the first trimester of pregnancy in Mexican American women living near the Texas–Mexico border and the incidence of NTDs in their babies. Fumonisin exposure was estimated in three ways: 1) maternal serum measurements of the sphinganine to sphingosine ratio, 2) maternal dietary recall of total maize tortilla intake during the first trimester of pregnancy and 3) imputed fumonisin exposure based on total tortilla intake and measurement of fumonisin levels in tortillas in 6-month blocks, to account for possible seasonality of fumonisin contamination. Specifically, FB_1 levels were reported in the study; the authors mentioned that FB_2 and FB_3 levels were essentially non-detectable in the tortilla samples. Although the sphinganine to sphingosine ratio has not been validated as a human biomarker of fumonisin exposure, this study did indicate a generally monotonically increasing relationship between the ratio of serum sphinganine to sphingosine concentrations and the adjusted odds ratios of NTDs in the population in seven dose groups, except at the highest dose (sphinganine/sphingosine >0.35). The authors explained that at the highest estimated fumonisin dose, the mothers may have miscarried their babies instead of giving birth to babies with NTDs. The implication is that there is a dose–response relationship between maternal fumonisin exposure and increased risk of NTDs in babies. Likewise, although maternal dietary recall of tortillas consumed in the first trimester did not correlate with odds ratios for NTD risk, the estimated fumonisin exposure based on tortilla samples did (except, again, at the highest estimated fumonisin dose: >650 ng/kg bw per day).

2.4.5 Human biomarkers of fumonisin exposure

Biomarkers are important in assessing dietary mycotoxin exposure because, until recently, human exposure to mycotoxins was measured almost exclusively in one of two ways: by questionnaires or food diaries relying on participants' recall of what and how much had been eaten; or by food samples collected from populations that ideally were representative of true exposures. Both of these methods pose potential problems. Dietary recall can often be inaccurate. It can be a challenge in

many cultures worldwide to take food samples for analysis without disturbing social contexts, and individuals' knowledge that their food is being analysed may lead to abnormal food preparation or eating behaviours during experiments (Hall & Wild, 1994). In recent years, however, biomarkers to assess mycotoxin exposure, internal dose and biologically effective dose have been developed and are increasingly being used to estimate human exposure (Groopman, Kensler & Wild, 2008). Biomarkers can also be used to assess the effectiveness of interventions to reduce mycotoxin exposure. It is important, however, to validate biomarkers before using them to assess either exposure to or the effect of different compounds.

Several studies have been conducted over the last decade in an attempt to find a reliable biomarker for fumonisin exposure in humans. These are reviewed in Shephard, Van der Westhuizen & Sewram (2007) and Cano-Sancho et al. (2010) and are summarized below.

(a) Sphingoid bases and their ratio

In animals, sphingoid bases (sphinganine and sphingosine) and the sphinganine to sphingosine ratio in plasma and urine have proven to be reliable biomarkers for fumonisin exposure. However, these are not validated biomarkers of fumonisin exposure in humans, as the studies that have attempted to link dietary fumonisin with sphinganine, sphingosine or their ratio in a variety of media have yielded inconsistent results.

Solfrizzo et al. (2004) measured mean fumonisin levels (FB_1 + FB_2) and the ratio of urinary sphinganine to sphingosine concentrations in two populations with high maize consumption (northern Argentina and southern Brazil, $n = 123$) and two populations with low maize consumption (central Argentina and southern Italy, $n = 66$). Indeed, the mean urinary sphinganine to sphingosine ratio was significantly higher in the high maize consumption areas (1.27) than in the low maize consumption areas (0.36) ($P < 0.001$). Within the two high-consumption areas, however, although the mean amounts of fumonisin exposure were similar, the mean urinary sphinganine to sphingosine ratio was significantly higher in southern Brazil (1.57) than in northern Argentina (0.69) ($P < 0.05$). Thus, the authors concluded that the urinary sphinganine to sphingosine ratio could not be associated with fumonisin exposure and that further studies were needed to provide convincing evidence of this ratio as a biomarker of human fumonisin exposure.

Another study conducted on 20 husband–wife pairs in Burkina Faso (Nikièma et al., 2008) showed varying results regarding the different sphingoid base measurements. FB_1 + FB_2 was measured in individuals' plate-ready food, whereas sphinganine and sphingosine concentrations were each measured in three different media: urine, buccal cells and serum. A modest positive trend was observed between mean fumonisin exposure and mean serum sphinganine to sphingosine ratio ($P = 0.067$). Moreover, when individuals were dichotomized based on the median fumonisin exposure, the serum sphinganine to sphingosine ratio was moderately higher in the high-exposure group (0.64 [0.54–0.75]) than in the low-exposure group (0.49 [0.41–0.59]) ($P = 0.044$). However, none of the other markers were correlated with fumonisin exposure based on plate-ready food measurements.

Two South African studies showed that actual fumonisin levels in individuals' diets did not correlate with sphinganine or sphingosine levels or sphinganine to sphingosine ratios in plasma and urine at a population level (Van der Westhuizen et al., 2008) or at an individual level (Van der Westhuizen et al., 2010a). Maize samples were analysed for FB_1 + FB_2 + FB_3 in Bizana and Centane, two magisterial areas in the former Transkei, South Africa. Additionally, plasma and urine samples from male and female subjects were analysed for sphinganine and sphingosine. Estimated fumonisin exposure was 5.8 ng/g bw per day in Bizana and 4.4 ng/g bw per day in Centane in 2000. Plasma sphinganine to sphingosine ratios were significantly lower ($P < 0.05$) in Bizana than in Centane, whereas urinary female and combined (male and female) sphinganine to sphingosine ratios were significantly higher ($P < 0.05$) in Bizana. However, there was no significant difference in mean total fumonisin levels in the maize (Van der Westhuizen et al., 2008). Van der Westhuizen et al. (2010a) characterized individual fumonisin exposure with weighed food records and fumonisin levels in maize in each household and again measured plasma and urinary sphinganine and sphingosine levels. Again, there was no association between these sphingoid bases, their ratio and actual fumonisin exposure in individuals. The authors concluded that these results negated the sphingoid bases as potential biomarkers of fumonisin exposure in humans.

(b) Hair fumonisins

Sewram et al. (2003) obtained hair samples from populations in Bizana, Butterworth and Centane districts in South Africa to analyse for FB_1, FB_2 and FB_3. These compounds were detected using HPLC-ESI-MS. Hair from Centane and Butterworth had mean FB_1 levels of 26.7 and 23.5 ng/g hair, respectively. FB_2 was detected only in Centane and in one sample in Butterworth, whereas no samples contained more than trace levels of FB_3. Hair fumonisin levels could represent long-term exposure to fumonisins; however, the fumonisin levels in these samples were not compared with actual levels of fumonisins in the maize in these districts.

(c) Urinary fumonisin B_1

In recent years, the urinary FB_1 biomarker has been developed and tested (Gong et al., 2008; Xu et al., 2010; Van der Westhuizen et al., 2011a) and emerged as a strong candidate biomarker of exposure in a study that tracked both fumonisin levels in food and urinary FB_1 levels in humans (Van der Westhuizen et al., 2011a). Gong et al. (2008) compared the maize tortilla consumption (categorized as high, medium or low) of 75 women in Morelos County, Mexico, with their urinary FB_1 levels. Urinary FB_1 level was correlated with maize intake ($P = 0.001$), a correlation that remained significant after adjusting for age, education and place of residence. Women with high maize tortilla intake had 3 times higher average urinary FB_1 levels compared with women in the low-intake group ($P = 0.0015$).

In a cross-sectional study in Huaian and Fusui counties, China (Xu et al., 2010), serum and urinary sphinganine and sphingosine concentrations were measured, along with urinary FB_1, in 43 Huaian adults and 34 Fusui adults. Most (40/43; 93.0%) of the Huaian subjects and 52.9% (18/34) of the Fusui subjects had estimated daily FB_1 exposures greater than the PMTDI of 2 µg/kg bw. At a

population level, mean estimated exposure to FB_1 did not correlate with any of the sphingoid base measurements or their ratio, but did correlate with urinary FB_1 level. In Huaian, the median urinary FB_1 level was 3.91 (0.06–254) ng/mg, whereas in Fusui, the median urinary FB_1 level was 0.39 (0.01–3.72) ng/mg. The authors suggested that urinary FB_1 could be a potential biomarker for fumonisin exposure.

Van der Westhuizen et al. (2011a,b) implemented a simple intervention of hand sorting and washing of maize to measure reductions in fumonisin levels in porridge and urinary FB_1 levels in 22 female participants in Centane, South Africa. Geometric mean FB_1 exposures through porridge consumption before and after the intervention were, respectively, 4.84 (2.87–8.14) and 1.87 (1.40–2.51) µg/kg bw per day. This correlated with reductions in urinary FB_1 normalized for creatinine before and after the intervention, which was reduced from 470 to 279 pg/mg creatinine. At the individual level, the urinary FB_1 normalized for creatinine biomarker was positively correlated with FB_1 exposure ($r = 0.4972$; $P < 0.01$). Urinary excretion of FB_1 was estimated to be about 0.075% of total FB_1 exposure. That the intervention could reduce the biomarker levels is a powerful indicator of a potentially validated biomarker for fumonisin exposure in humans. However, the authors confirmed that more studies are needed in larger populations worldwide to confirm these analyses.

3. ANALYTICAL METHODS

3.1 Chemistry

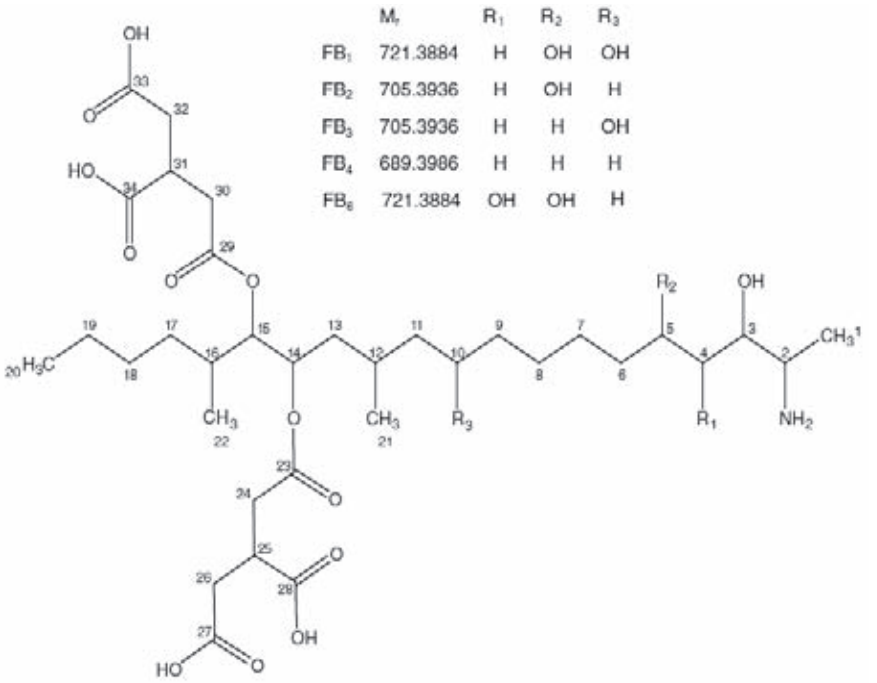
The B series of the fumonisins (FB_1, FB_2, FB_3 and FB_4), the major forms found in food, were previously described by the fifty-sixth meeting of the Committee (Annex 1, reference *153*). Fumonisins do not have a cyclic structure (Figure 3). They are characterized by a 19- or 20-carbon aminopolyhydroxyalkyl chain diesterified with propane 1,2,3-tricarboxylic acid groups (tricarballylic acid) (Jackson & Jablonski, 2004).

The fumonisin analogues can be classified into four main groups, A, B, C and P series. Since the fifty-sixth meeting of the Committee (Annex 1, reference *153*), of the forms that have been characterized, the number of known fumonisin analogues has greatly increased (Gelderblom et al., 2007).

The A series of fumonisins, isolated from cultures of *Fusarium verticillioides* and whole corn, has an *N*-acetyl amide group at the C-2 position. The C series, which has been isolated from mouldy corn, is chemically similar to the B series, except that the C-1 terminal methyl group is missing in this series (Jackson & Jablonski, 2004).

Sewram et al. (2005) showed the ability of *F. verticillioides* and *F. proliferatum* to simultaneously produce fumonisin B and fumonisin C series analogues. Isolates that produced fumonisin B analogues produced at least 10-fold more of the B series analogues than they did of the C series analogues. Their results confirm that at least some strains of *F. oxysporum* produce fumonisin C, but not fumonisin B, analogues.

Figure 3. Chemical structures of FB_1 (CAS No. 116355-83-0), FB_2 (CAS No. 116355-84-1), FB_3 (CAS No. 136379-59-4), FB_4 (CAS No. 136679-60-7) and FB_6



The P series of fumonisins, isolated from cultures of *F. proliferatum* grown on corn, contain a 3-hydroxypyridinium moiety at the C-2 position in the backbone instead of the amine found in the B series (Jackson & Jablonski, 2004).

Bartók et al. (2006) detected 37 new fumonisins and grouped them according to fumonisin types: fumonisin B, fumonisin A, fumonisin C and a new series type, FBX. The FBX series is esterified by other carboxylic acids, such as *cis*-aconitic acid, oxalylsuccinic acid and oxalylfumaric acid.

Four new compounds (belonging to the FBX series described above) were detected by Bartók et al. (2008). In these compounds, one of the hydroxyl groups attached to the fumonisin backbone was esterified by a carboxylic acid (oxalylsuccinic acid) other than tricarballylic acid.

Some of these analogues may occur in naturally contaminated maize at relatively low levels (<5% of the sum of FB_1, FB_2 and FB_3 levels), and they are not detected with most traditional analytical methods due to the derivatization process, but can be detected with the use of LC-MS (Rheeder, Marasas & Vismer, 2002; Bartók et al., 2006).

New components described by Bartók et al. (2010a) are significantly more apolar than earlier-described fumonisins, and their uptake into and toxicity elicited

in the various tissues of living organisms may therefore be different from those of other mentioned fumonisins. The new compounds were denoted as esterified FB_1 (EFB_1) toxins, with the suggested names EFB_1 PA, *iso*-EFB_1 PA, EFB_1 LA, *iso*-EFB_1 LA, EFB_1 OA and *iso*-EFB_1 OA, where the fumonisin backbone is esterified by the fatty acids $C_{16}H_{32}O_2$ (palmitic acid, PA), $C_{18}H_{32}O_2$ (linoleic acid, LA) and $C_{18}H_{34}O_2$ (oleic acid, OA). The total amount of these new compounds is estimated to comprise 0.1% of the FB_1 concentration.

The FB_1 toxin has 10 chiral centres; theoretically, therefore, 1024 stereoisomers can be produced. The 28 FB_1 stereoisomers found by Bartók et al. (2010b) represented 2.8% of the quantity of FB_1.

Analysis of the analytical standard of FB_3 obtained from different batches of *F. verticillioides* has recently shown the presence of a stereoisomer of FB_3 at 10–40%. The identification and absolute configuration of the stereoisomers 3-*epi*-FB_3 and 3-*epi*-FB_4 were reported by Gelderblom et al. (2007).

Fusarium verticillioides and *F. proliferatum* are the main sources of fumonisins in maize. Other fumonisin-producing species are *F. nygamai*, *F. napiforme*, *F. thapsinum*, *F. anthophilum* and *F. dlamini* from millet, sorghum and rice (Frisvad et al., 2006).

Aspergillus niger is a common fungus growing in grapes, green coffee beans, onions, mango, corn and other cereals, peanuts and dried fruits, and it was shown that it is able to produce FB_1, FB_2 and FB_4 (Frisvad et al., 2007; Nielsen et al., 2009; Varga et al., 2010). Recently, Mansson et al. (2010) isolated a new fumonisin, FB_6, together with FB_2, from stationary cultures of the fungus *A. niger.* Analysis of MS and NMR data determined that FB_6 is a positional isomer of FB_1 and *iso*-FB_1, having hydroxyl functions at C-3, C-4 and C-5.

Several authors demonstrated the presence of bound fumonisins, but there is confusion about the terminology or classification used for the different fractions of fumonisins found in foods and feeds. One of the recent classifications is the one suggested by Berthiller et al. (2009), who proposed the presence of free fumonisins and conjugated fumonisins, either soluble (called "masked" mycotoxins) or associated with/attached to macromolecules through covalent linkages (called "bound" mycotoxins). These conjugated mycotoxins can emerge after metabolism by living plants, fungi and mammals or after food processing. A second classification adopted by Dall'Asta et al. (2009b) divides fumonisin amounts after different treatments into:

- *Extractable fumonisins*: the sum of FB_1, FB_2 and FB_3 found in each sample upon application of common extraction conditions;
- *Total fumonisins*: the sum of FB_1, FB_2 and FB_3 found in each sample after hydrolysis; this value is actually obtained by measuring HFB_1, HFB_2 and HFB_3 and then converting the data into fumonisin B equivalents by means of the proper conversion factor;
- *Hidden fumonisins*: the difference between total fumonisins and extractable fumonisins.

These authors suggest that the term "hidden fumonisin" should be used only for non-covalently bound derivatives, which are formed through an associative interaction between fumonisins and matrix macroconstituents, and the term "bound fumonisin" should be used only for those compounds that involve covalent linkages among the analyte and the matrix constituents. The linkages may involve fumonisin free amino groups or the carboxylic moieties. This division is more related to the analytical methods currently used for quantification and also takes into account not only that fumonisins are bound through covalent linkages to other molecules, but also that there are other mechanisms, such as complexation or physical entrapment, different from covalent binding, that could hide fumonisins. Therefore, the Committee has decided to use this terminology, bearing in mind that the exact chemical nature of the occurring masked forms is still not clear, and further investigations should be carried out in order to get a full picture of the bound and hidden fumonisin issue.

3.2 Chemical analysis

3.2.1 Screening tests

Screening methods have found wide application in mycotoxin analysis because they provide rapid and sensitive detection and are cost-effective. They are easy to use and so can be applied by non-specialists both in the laboratory and under field conditions. Quantification of the toxin is not always necessary, and a simple presence/absence test based on visual evaluation is sufficient. In this regard, different visual labels, such as enzymes for catalytic enzymatic reactions, colloidal gold, fluorescent labels and liposomes encapsulating a visible dye, are used. The cut-off level can be established based on either noticeable reduction of colour development or complete colour suppression. Some tests assume semiquantitative estimation based on colour intensity. To make interpretation of results easier, special control zones have been included in most tests. All rapid tests with visual estimation are combined with a simple sample preparation procedure, which usually includes extraction with methanol or methanol/water (or buffer) mixture, filtering and dilution with buffer (Goryacheva et al., 2007).

The fifty-sixth meeting of the Committee reviewed the use of TLC, ELISA and other immunologically based methods such as dipsticks and biosensor methods, as well as the use of immunoaffinity columns in direct fluorometric methods.

Since the last review, a number of immunoassays and antibodies have been developed for the determination of fumonisins, and several manufacturers now offer test kits based upon several platforms, such as ELISA or immunochromatographic (lateral flow) devices. These have been reviewed by Goryacheva et al. (2007). Some of these ELISAs and lateral flow devices have had their performance verified by third-party organizations. Web sites of AOAC International (http://www.aoac.org) and the United States Department of Agriculture's Grain Inspection, Packers and Stockyards Administration (http://www.gipsa.usda.gov) may be consulted to determine which fumonisin test kits have had their performance verified (Shephard et al., 2010). Lateral flow tests, also known as immunochromatographic strip tests, are unique, rapid and user-friendly test formats that do not require instruments or additional handling steps. They are available in several forms, depending upon

which reagent is labelled (the antigen or the antibody) and the type of the label. The most popular label for the immunochromatographic strip test is colloidal gold (Goryacheva et al., 2007). Recently, a membrane-based colloidal gold immunoassay lateral flow test for the rapid detection of FB_1 (Wang et al., 2006) was extended to determine the sum of FB_1, FB_2 and FB_3, with applicability to the analysis of naturally contaminated maize. The assay time was reported as 4 minutes (Molinelli, Grossalber & Krska, 2009).

Immunoassays are now widely used for fumonisin detection, especially in industrial applications. The application of test strips to mycotoxins, including fumonisins, was recently reviewed by Krska & Molinelli (2009). Flow-through or immunofiltration assay has also been used for fumonisins in maize at a detection limit of 1000 ng/g (Paepens et al., 2004). Contrary to earlier immunofiltration assays (Schneider, Usleber & Märtlbauer, 1995), where the limit of detection (LOD) for FB_1 was determined as colour intensity reduction, the LOD in this case was determined as complete colour suppression. Immunoassays are cheap, fast, portable and suitable for routine screening of samples, but have limitations in selectivity and reproducibility. Results obtained therefore need to be confirmed using reference quantitative methods.

Many laboratories in developing countries still rely on TLC as the primary methodology for mycotoxin determination. A simple and cost-effective semiquantitative method using TLC was developed for FB_1 in maize (Shephard & Sewram, 2004). Extraction was by shaking with methanol/water (75:25) for 60 minutes followed by cleanup on a strong anion exchange (SAX) cartridge. The purified residue was reacted with fluorescamine, and the FB_1 derivative was separated by reversed-phase TLC and visualized as a greenish-yellow spot under long-wavelength ultraviolet (UV) light alongside spotted fumonisin standards. The method was collaboratively studied in 14 laboratories. The within-laboratory relative standard deviation (RSD) ranged from 27.1% to 41.7%, and the between-laboratory RSD was from 35.0% to 63.3%. The mean recovery was 75% at a spiking level of 2.00 mg/kg.

The current trend is the development of multi-mycotoxin screening methods. In this regard, a prototype eight-well immunofiltration test device has been developed for the simultaneous determination of seven mycotoxins, including FB_1 (Schneider et al., 2004). The assay is similar to one for single-mycotoxin determination, except that a mixture containing the respective toxin–enzyme conjugates was used for all wells. The detection level for FB_1 in spiked wheat was 50 ng/g. A disadvantage of multi-analyte tests with visual detection is the loss of sensitivity when compared with single-analyte determination (Goryacheva et al., 2007).

All the procedures described above may be classified as non-instrumental rapid screening techniques that could be used outside the laboratory environment or at the place of sampling. To obtain quantitative or semiquantitative results, immunochemical methods based on relatively simple equipment, such as microplate reader, luminometer or capillary electrophoresis system, have been developed and may be used (Goryacheva et al., 2007). The microtitre plate ELISA format is commonly used as a rapid test for mycotoxins, including fumonisins. They are commercially available and have benefits of speed and sensitivity. ELISAs,

however, generally overestimate the concentration of fumonisins present in samples as a result of antibodies' cross-reactivity to compounds that are structurally related to fumonisins (Bird et al., 2002; Jackson & Jablonski, 2004). A few developments regarding the use of biosensor or surface Plasmon resonance sensor systems and fluorescence polarization immunoassay for fumonisins are available (Maragos et al., 2001; Ligler et al., 2003; Van der Gaag et al., 2003; Sapsford et al., 2006), but have not found widespread application.

3.2.2 Quantitative methods

The fifty-sixth meeting of the Committee reviewed the quantitative methods available for the analysis of FB_1, FB_2 and FB_3 in maize and maize-based food matrices using HPLC after cleanup on SAX or C_{18} solid-phase extraction (SPE) cartridges. This HPLC method was based on fluorescence detection with pre-column derivatization with *o*-phthaldialdehyde and has subsequently been adopted by AOAC International as an official method (AOAC Method 995.15) for maize analysis (Sydenham et al., 1996). This method, however, showed low recoveries and fluorescent interferences by matrix components, as well as high variability when applied to more complex maize-based food matrices, such as corn flakes, muffins and other processed maize products (Scott & Lawrence, 1996; De Girolamo et al., 2001; Paepens et al., 2005b).

Since the fifty-sixth meeting of the Committee, the most significant advances in mycotoxin analysis have been the application of LC methods coupled to MS detection systems and the use of MS-based methods for multi-mycotoxin determination. Nevertheless, pre-column derivatization of fumonisins with *o*-phthaldialdehyde followed by LC and fluorometric detection continues to be extensively used for fumonisin detection (Shephard et al., 2009). One reason is that not all laboratories can afford to purchase mass spectrometers. Purification of extracts to remove matrix impurities and to concentrate fumonisins is achieved by using SPE, reversed-phase (C_{18}) or SAX cartridges or immunoaffinity columns. SAX columns provide cleaner extracts for maize but require monitoring of pH of the sample above 5.8. In addition, the elution flow rate should not be higher than 1 ml per minute for adequate retention (Sydenham, Shephard & Thiel, 1992).

Fumonisins do not fluoresce, nor do they contain UV-absorbing chromophores. Consequently, most HPLC methods measure fumonisins after derivatizing (pre-column) the free amino group with fluorescent compounds. The most commonly used reagent is *o*-phthaldialdehyde (Shephard et al., 1990; Ross et al., 1991; Pagliuca et al., 2005). The *o*-phthaldialdehyde derivatives of fumonisins have limited stability and need to be analysed immediately. This problem may be overcome by standardizing the time (2 minutes) between reagent addition and HPLC injection. Recent studies have also shown that *o*-phthaldialdehyde-derivatized samples are stable for at least 24 hours at 4 °C (Williams, Meridith & Riley, 2004).

To overcome the problem of limited stability of *o*-phthaldialdehyde derivatives, LC-fluorescence of FB_1 and FB_2 with post-column derivatization is now available and has been automated (Muscarella et al., 2008). The method was developed for the quantitative determination of FB_1 and FB_2 in maize-based foods for direct human consumption and uses HPLC-fluorescence with a rapid and automated

online post-column derivatization, performed with *o*-phthaldialdehyde and *N*,*N*-dimethyl-2-mercaptoethylamine. Optimal fluorescence detection was obtained by using excitation and emission wavelengths of 343 nm and 445 nm, respectively. Fumonisins were separated in less than 13 minutes using a C_{18} column and a gradient elution at 0.8 ml per minute with methanol and 0.1 mol/l phosphate buffer at pH 3.15. The LODs for FB_1 and FB_2 were 5 and 6 µg/kg, respectively. Recovery values ranged from 87% to 94% for FB_1 and from 70% to 75% for FB_2 in corn flake samples at three fortification levels in the range 100–300 µg/kg. The method has been successfully applied to maize flour, "polenta", tortillas and cookies (Muscarella et al., 2008).

Other derivatization reagents available but not highly patronized include 4-fluoro-7-nitrobenzofurazan (Scott & Lawrence, 1992), naphthalene-2,3-dicarboxaldehyde (Bothast et al., 1992; Scott & Lawrence, 1994; Lino et al., 2006), 4-(*N*,*N*-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (Akiyama et al., 1995) and fluorescamine (Murphy et al., 1996).

An adaptation of the HPLC-fluorescence method was the use of the fluorescent label (*o*-phthaldialdehyde), but monitoring the UV absorbance of the product rather than the fluorescence (Ndube, Van der Westhuizen & Shephard, 2009). This study was directed at laboratories equipped with HPLC and UV detectors rather than fluorescence detectors. Comparison of the detection systems using fumonisin standards indicated that fluorescence detection is about 20 times more sensitive than UV detection. When applied to maize samples with FB_1 levels above 1000 µg/kg, the two detection systems were comparable and gave repeatabilities equal to or less than 10%, suggesting an alternative in certain circumstances.

A comparison between HPLC-MS, HPLC-MS/MS and an HPLC-fluorescence technique for fumonisins in maize has been described (Silva et al., 2009). Maize products were extracted with aqueous methanol, and fumonisins were isolated by immunoaffinity column cleanup and detected using each of the three methods. The LC fluorescence method used naphthalene-2,3-dicarboxaldehyde as the labelling reagent. The limits of quantification (LOQs) for FB_1 and FB_2 were 12 µg/kg for HPLC-MS and 40 µg/kg for HPLC-MS/MS. LOQs were 15 µg/kg and 20 µg/kg for FB_1 and FB_2, respectively, when HPLC-fluorescence was used. Recoveries ranged from 79% to 102% for FB_1 and FB_2, with an RSD ranging from 9% to 17% at spiking levels of 150 and 250 µg/kg.

Ofitserova et al. (2009) combined LC with post-column photochemical and chemical derivatization for the detection of multiple toxins, including DON, aflatoxins, OTA, ZEA, FB_1 and FB_2. The method used a single methanol–water extraction. Aflatoxins, OTA, ZEA and fumonisins were isolated using immunoaffinity columns, whereas a Mycosep column was used for DON. The purified extracts were pooled together before injection onto the LC. The procedure was evaluated by subjecting it to the AOAC International criteria for a single-laboratory validation. Good recoveries and LODs were observed.

Despite the wide use of immunoaffinity columns and their general acceptance for the isolation of mycotoxins from complex matrices as a prelude to HPLC-fluorescence or HPLC-MS methods, high recoveries should not be

assumed. Studies by Oh, Scott & Chung (2009) have shown incomplete recovery of fumonisins from naturally contaminated maize when methanol alone was used for elution. Methanol–water (80:20) has therefore been recommended (Oh, Scott & Chung, 2009).

Some advances have also been made in the use of SPE cleanup columns. Recently, De Smet et al. (2009) reported that a synthetic receptor, molecularly imprinted polymer, produced for fumonisin B analogues was incorporated in an SPE column for isolation of fumonisins from corn flakes. Recoveries for the molecularly imprinted SPE procedure when applied to corn flakes ranged from 62% to 72% for FB_1; from 71% to 75% for FB_2; and from 65% to 70% for FB_3. Analysis of 39 naturally contaminated corn flake samples by LC-MS/MS indicated that the synthesized molecularly imprinted polymer could be a good alternative for cleanup and preconcentration of fumonisins in food samples. The study also showed that the developed molecularly imprinted polymer could be reused up to 50 times.

Most chromatographic methods are based on the determination of single or multiple mycotoxins belonging to the same group due to the chemical diversity of the different toxin classes, such as acidic (fumonisins), basic (ergot alkaloids), polar (MON) and apolar (ZEA, BEA) compounds, and this has hampered multi-mycotoxin analysis (Krska et al., 2008). In general, these methods require analysis times longer than 20 minutes (Cavaliere et al., 2005; Spanjer, Rensen & Scholten, 2008), and in some cases, two injections are required (Sulyok et al., 2006), as well as the application of a cleanup step (Cavaliere et al., 2005).

The co-occurrence of different toxins has raised concerns about health hazards from contaminated food and feed (Creppy et al., 2004; Speijers & Speijers, 2004). There is also the need to cover the toxins addressed by European Commission Regulation 1881/2006 (AFB_1, AFB_2, AFG_1, AFG_2 and AFM_1, OTA, patulin, DON, ZEA, FB_1 and FB_2, HT-2 and T-2 toxin) with a single method, as this increases sample throughput and decreases the cost per analysis (Krska et al., 2008). These demands have resulted in a trend towards the use of MS-based methods for fumonisin analysis, because they allow for multi-mycotoxin determinations, most often without immunoaffinity cleanup or derivatization steps (Cirillo et al., 2003b; Sforza, Dall'Asta & Marchelli, 2006; Zoellner & Mayer-Helm, 2006; Spanjer, Rensen & Scholten, 2008). Since the fifty-sixth meeting of the Committee, several multi-mycotoxin procedures have been developed for the simultaneous determination of as many as 38 mycotoxins, including FB_1, FB_2 and HFB_1 (Sulyok, Krska & Schuhmacher, 2007b). The multi-mycotoxin assays using LC-MS have recently been reviewed by Songsermsakul & Razzazi-Fazeli (2008).

MS offers sufficient selectivity (especially if tandem MS is applied). LC-based methods using several analysers, such as single quadruple (Silva et al., 2009) or tandem MS (MS/MS), are more frequently used (Cavaliere et al., 2005; Faberi et al., 2005; Sulyok et al., 2006; D'Arco et al., 2008; Spanjer, Rensen & Scholten, 2008; Frenich et al., 2009; Silva et al., 2009). The LC-MS/MS methods vary tremendously, from "dilute and shoot" methods to those incorporating isolation steps (immunoaffinity columns or other SPE cleanup columns) to reduce matrix effects. The methods are also dependent on the number of analytes, which may vary from a few up to 87 (Shephard et al., 2009). LC-MS/MS methods have been developed

for fumonisins in a large number of matrices, and these have been summarized by Shephard et al. (2009). The matrices include wheat beer and sake (Rudrabhatla & Wood, 2007), maize (Lattanzio et al., 2007), maize meal (Cavaliere et al., 2007), cattle forage (Huls, Zuiderent & Ghosh, 2007), bread crumbs and mouldy foods (Sulyok, Krska & Schuhmacher, 2007a), spelt, rice and barley (Sulyok, Krska & Schuhmacher, 2007b), peanut, pistachio, wheat, maize, corn flakes, raisins and figs (Spanjer, Rensen & Scholten, 2008) and rice (Kushiro et al., 2007).

Despite the increasing use of LC-MS or LC-MS/MS for multi-mycotoxin analysis, only one interlaboratory validation study is available for mycotoxins (FB_1 and FB_2) in food (Senyuva, Gilbert & Stroka, 2010). This method was validated by 12 laboratories in 11 countries. Extraction was with acetonitrile–methanol–water (25:25:50) followed by filtration and dilution with phosphate-buffered saline solution and cleanup with immunoaffinity column. FB_1 and FB_2 were eluted with methanol followed by water, then directly determined by reversed-phase LC with MS detection using selected-ion monitoring of two characteristic ions in each case. RSD values for within-laboratory repeatability (RSD_r) were 4.6–11.9%, 1.9–12.6% and 1.4–11.5% for FB_1, FB_2 and combined FB_1 + FB_2, respectively. Corresponding RSD values for between-laboratory reproducibility (RSD_R) were 19.8–23.8%, 18.2–25.5% and 18.8–23.2%. Horwitz ratio values for *r* and *R* were all less than 2, indicating that the method is suitable as a regulatory method for the enforcement of limits for fumonisins in maize. The results were consistent with previously reported data from a single-laboratory validation of the same method (Senyuva & Ozcan, 2008).

De Girolamo et al. (2010) subjected a method for the determination of FB_1 and FB_2 in maize-based baby food products to a limited validation study involving three laboratories. Determination was by HPLC with fluorimetric detection after immunoaffinity column cleanup. Mean recoveries ranged from 83% to 97% for FB_1 and from 62% to 78% for FB_2. The RSD for within-laboratory repeatability (RSD_r) ranged from 5% to 12% for FB_1 and from 8% to 13% for FB_2. The RSD for between-laboratory reproducibility (RSD_R) ranged from 6% to 10% for FB_1 and from 9% to 16% for FB_2. The LOQs were 2.8 µg/kg for FB_1 and 2.2 µg/kg for FB_2.

There are currently two official methods of AOAC International for the determination of fumonisins by HPLC. These are AOAC Method 995.15 (Sydenham et al., 1996) and AOAC Method 2001.04 (Visconti, Solfrizzo & De Girolamo, 2001).

Several analytical methods are now available for the determination of fumonisins in vegetable matrices, but very few methods exist for animal tissues. Currently, only three methods are available for quantifying fumonisins and their hydrolysed metabolites in animal matrices (Pagliuca et al., 2005; Fodor et al., 2008; Gazotti et al., 2011). The method of Gazotti et al. (2011) used LC-MS/MS for the analysis of FB_1, FB_2 and their hydrolysed metabolites in pig liver. Prior to LC-MS/MS, fumonisins were extracted into methanol–water (80:20) followed by SPE purification. Chromatographic separation was performed on a C_{18} column using 0.3% formic acid in water and acetonitrile as elution solvents. The method showed good performance characteristics, with an LOD of 0.05 µg/kg and an LOQ of 10 µg/kg for all the analytes. Silva et al. (2010) also reported an LC-MS/MS method for the determination of FB_1 and FB_2 in human urine.

A major problem encountered with MS methods is ion suppression (or enhancement) by residual food matrix resulting from inadequate cleanup. This may be dealt with through the use of cleanup steps, matrix-matched external standards or the use of internal standards (Beltrán et al., 2009; Di Mavungu et al., 2009; Frenich et al., 2009; Rasmussen et al., 2010). Isotopically labelled standards have shown great promise in adjusting for matrix effects in LC-MS (Shephard et al., 2009). These have been reviewed by Rychlik & Asam (2008).

LC-MS/MS methods in fumonisin analysis have advantages, which include low LOQs (low microgram per kilogram range) and the elimination of the need for fluorescent derivatives. Another method that has been applied to maize-based food samples that does not require derivatization is the use of LC coupled with an evaporative light scattering detector (Wang, Zhou & Wang, 2008). This method had the additional advantage of lower instrumentation cost, but with substantially higher LOQs than for the LC-MS methods. The LOQ was 3 µg/kg for components in solution.

Several additional new methods have been developed in the recent past for the detection of multiple mycotoxins or metabolites. These have been reviewed by Shephard et al. (2011). The methods include an LC-ESI-time of flight and an LC-ESI-ion trap method for detecting FB_1 and 28 stereoisomers (Bartók et al., 2010b), an LC-MS/MS assay using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged and Safe) approach to isolate 27 secondary metabolites from maize silage (Rasmussen et al., 2010), although validation results for the fumonisins were relatively poor, an LC-MS/MS method for the detection of 23 toxins in feed samples (Monbaliu et al., 2010), a multi-mycotoxin assay for mouldy foods (Sulyok, Krska & Schuhmacher, 2010) and an assay for 186 bacterial and fungal metabolites in indoor matrices, such as house dust (Vishwanath et al., 2009).

To increase the efficiency of chromatographic separations, ultra high-performance liquid chromatography (UPLC) is in use. This technology, coupled to MS/MS, has been applied to multiple mycotoxins in maize, pasta and baby food (Beltrán et al., 2009), a multi-toxin assay for fumonisins in beer (Romero-González et al., 2009), *Fusarium* mycotoxins in cereals (Zachariasova et al., 2010), mycotoxins, including fumonisins, in food supplements (Di Mavungu et al., 2009) and a multi-toxin assay for fumonisins in maize, walnuts, biscuits and breakfast cereals (Frenich et al., 2009). In the study by Beltrán et al. (2009), changes in chromatographic conditions significantly reduced the separation time of 11 mycotoxins to 4 minutes. Li, Herrman & Dai (2010) also developed a simple and robust method for the determination of FB_1, FB_2 and FB_3 in corn-based human food and animal feed using UPLC/ESI-MS/MS. A single-laboratory validation was conducted by testing three different spiking levels for accuracy and precision. Recoveries of FB_1 ranged from 93% to 98%, with RSD values of 3–8%. For FB_2, recoveries were from 104% to 108%, with RSD values of 2–6%. Recoveries for FB_3 were from 94% to 108%, with RSD values ranging from 2% to 5%. The LOD values for FB_1, FB_2 and FB_3 were 30, 20 and 6 µg/kg, respectively. LOQ values obtained for FB_1, FB_2 and FB_3 were 100, 50 and 20 µg/kg, respectively.

Current problems relating to analytical methods include the lack of suitable reference materials for method validation and the unavailability of standards, particularly for the hydrolysed fumonisins (HFB_1, HFB_2, HFB_3) and FB_6.

3.2.3 Quantitative methods for hidden and bound fumonisins

In the past few years, several publications have demonstrated the presence of fumonisins potentially bound to or associated with proteins or other food components, not only through covalent linkage to other molecules, but also through other mechanisms, such as complexation or physical entrapment. These hidden and bound forms escape conventional analysis and can be determined only in an indirect way—for example, through the application of a hydrolysis step (Kim, Scott & Lau, 2003; Park et al., 2004; Dall'Asta et al., 2008).

Methods for the determination of FB_1, FB_2 and FB_3 involve in most cases HPLC separation or HPLC-MS detection after solvent extraction from maize and maize-based food matrices. After the extraction of these free forms, a residue is left that can be subjected to alkaline hydrolysis in order to determine bound fumonisins.

Kim, Scott & Lau (2003) analysed the presence of protein-bound fumonisins in corn flakes. For that purpose, samples were first extracted with methanol:acetonitrile:water (25:25:50 by volume), and the residue was treated with 1% sodium dodecyl sulfate (SDS) and hydrolysed with 2 N potassium hydroxide. Protein-bound fumonisin was determined as HFB_1 by HPLC and confirmed by MS with ESI. The mean level of recovery for HFB_1 was 63.2%, and the LOD was 10 µg/kg.

For quantification of protein-bound FB_1 and total bound FB_1 (fumonisins bind to other molecules besides proteins) in corn foods, Park et al. (2004) developed a cleanup procedure so that the existence of these forms could be confirmed by HPLC-MS of the HFB_1. For the analysis of protein-bound FB_1, these authors used the method described above by Kim, Scott & Lau (2003), washing the solid residue with methanol:acetonitrile:water (25:25:50 by volume) and extracting with 1% SDS by shaking. To remove the remaining SDS from the protein-bound fumonisin to improve the recovery, 1% methylene blue was used for complexation, followed by solvent extraction and hydrolysis with 2 N potassium hydroxide. To measure total bound FB_1, the sample itself was hydrolysed with potassium hydroxide. In both cases, cleanup was accomplished on an OASIS polymeric SPE column, the bound fumonisins were determined by HPLC measurement of HFB_1 and confirmation was done with tandem MS with ESI. Recoveries for HFB_1 ranged from 71% to 104% at 200 µg/kg, depending on the food matrix, and the LOD was 1 µg/kg.

In accordance with the alkaline treatment, Dall'Asta et al. (2008) proposed a method for simultaneous quantification of free and bound fumonisins in corn and corn-based products. This involved extracting with methanol:acetonitrile:water (25:25:50 by volume), blending and filtering for free fumonisins and for bound fumonisins, washing the residue with the same solvent, drying and placing the solid residue in sodium hydroxide (2 mol/l) for 60 minutes at 25 °C for its hydrolysis. The aqueous phase was then extracted with ethyl acetate, and the organic phase was evaporated. The residue was finally dissolved in water:acetonitrile (1:1 by volume), filtered through a nylon filter and analysed by HPLC-MS. The amount of HFB_x is a measurement of bound fumonisin. The recovery was higher than 90% in all cases at 500 µg/kg, and the LODs were 1 µg/kg for FB_1 and FB_2, 8 µg/kg for FB_3, 20 µg/kg

for HFB_1 and HFB_3 and 25 µg/kg for HFB_2. Other methods proposed by Dall'Asta et al. (2009a) to quantify bound fumonisin used the alkaline treatment and calculated the amount of total bound fumonisin indirectly by subtracting the amount of free FB_x and HFB_x found before the hydrolysis from the total fumonisin content.

Hidden fumonisins, as described above, were calculated by Dall'Asta et al. (2009b) in raw maize by subtracting the amount of extractable fumonisin from total fumonisin.

Recent studies performed by Gazzotti et al. (2011) showed that chromatographic separation of FB_1, FB_2, HFB_1 and HFB_2 from animal liver tissue samples could be performed using HPLC with a C_{18} column with 0.3% formic acid in water and acetonitrile as elution solvent and quantified with MS operated in ESI+ using multiple reaction monitoring. The LOD for all the fumonisins was 0.05 µg/kg, and the trueness of the method ranged from –0.9% to 0.2%.

Motta & Scott (2009) proposed an in vitro digestion model to determine total bound fumonisin in corn flakes. In this experiment, the digestion process in the human gastrointestinal tract was simulated by adding artificial digestive fluids to corn flakes at a certain pH, temperature and residence time periods. After hydrolysis of the chyme with potassium hydroxide, a cleanup extract OASIS HLB extraction column was used, and total bound FB_1 was determined as HFB_1 with HPLC. The recovery for HFB_1 at 437 µg/kg was 92%. Dall'Asta et al. (2010) also analysed for the occurrence of hidden fumonisins in maize samples with this digestion approach, finding that an increased amount of total detectable fumonisins was observed in comparison with the analysis on the non-digested matrix.

In contrast, some authors have reported that FB_1 decreases during heat treatment and long cooking, depending on temperature, exposure time, contaminant level and level of a reducing sugar, *N*-(carboxymethyl) FB_1, one of the principal products of the Maillard reaction between FB_1 and glucose (Seefelder, Knecht & Humpf, 2003; Meca et al., 2010). In a study performed by Meca et al. (2010), a method for the determination of FB_1 and *N*-(carboxymethyl) FB_1 in corn was proposed. In this case, a high-speed blender, Ultra-Turrax, was used to homogenize the samples with methanol:water (70:30 by volume) as a solvent for extraction, and separation and quantification were done with LC-MS/MS.

Nevertheless, most of the currently used analytical methods are not able to reveal the occurrence of intact fumonisins hidden in the matrix. The occurrence of higher levels of fumonisins in reference material after digestion showed that the reference material is a major point to be considered during method validation. Moreover, the lack of available standards to improve and develop new methodologies also raises a problem, as well as the lack of knowledge of the entire reactions surrounding the binding phenomena. Further studies are required in order to get more appropriate methodologies for the determination of fumonisins.

4. SAMPLING PROTOCOLS

To obtain representative data for the different analytical methodologies, the sampling stage has to be as representative as possible. It is well known that the

distribution of mycotoxins among food and feed is quite heterogeneous, so the sampling stage of the overall mycotoxin contamination can lead to great variation in the results.

Previous work on sampling plans for fumonisins described by the fifty-sixth meeting of the Committee (Annex 1, reference *153*) showed that at a batch fumonisin concentration of 2 mg/kg, the coefficient of variation associated with the total test procedure (sampling, sample preparation and analysis) was 45%.

For the purpose of official control of the levels of mycotoxins in foods, the European Commission has sampling protocols, including for fumonisins, that regulate the number and size of the incremental samples and size of the aggregate sample to be taken (EC, 2006) and were applied in several studies included in the occurrence tables in Appendix 1. Nevertheless, at least in the case of fumonisins, all possible aspects are not covered by these sampling protocols. Different approaches have been used in recent years to improve the sampling stage of a given protocol.

A sampling study was designed by Whitaker et al. (2007) to determine the variability and distribution among sample test results when food-grade maize marketed in Nigeria was sampled for fumonisin. In total, 86 food-grade maize lots intended for human consumption were sampled. On average, 17 test samples were taken from each lot, 100 g of each were finely ground using a RAS II Romer mill and 25 g of subsample was analysed for FB_1 by HPLC. The total variability associated with the fumonisin test procedure was measured for each lot. Regression equations were developed to predict the total variance as a function of fumonisin concentration, which was compared with several theoretical distributions. The negative binomial distribution was selected to model the fumonisin distribution among test results. A computer model was also developed to predict the performance of sampling plan designs in detecting fumonisin in maize samples.

The total variability associated with a fumonisin test procedure that used 100 g samples and 25 g subsamples with an HPLC analytical method was found to be a function of fumonisin concentration in the lot. The variance, standard deviation and coefficient of variation among 100 g samples used to detect fumonisin in a shipment of maize with a true concentration of 2 µg/g were 1.91%, 1.38% and 69%, respectively. The uncertainty associated with the fumonisin test procedure used to detect fumonisin in maize marketed in Nigeria was similar in magnitude to the uncertainty of the fumonisin test procedure used to detect fumonisin in shelled corn marketed in the south-eastern USA.

Johansson et al. (2006) conducted a study to determine whether fumonisins are concentrated in the poor-quality grade components of shelled corn. Four 1.0 kg test samples were taken from each of 23 lots of shelled corn marketed in North Carolina, USA, and each test sample was divided into three grade components: 1) damaged kernels, 2) broken corn and foreign material and 3) whole kernels. Fumonisin concentration was measured in each component, and a mass balance equation was used to calculate the total concentration in each test sample. Averaged across all test samples, the fumonisin concentrations in the damaged kernel, broken corn and foreign material, and whole kernel components were 148.3, 51.3 and 1.8 mg/kg, respectively. The damaged kernel and broken corn and

foreign material components combined accounted for only 5.0% of the test sample mass, but accounted for 77.5% of the total fumonisin mass in the test sample. In the combined damaged kernel and broken corn and foreign material components, high correlations with fumonisin concentration in the lot were found. This study indicated that measuring fumonisin in the combined damaged kernel and broken corn and foreign material grade components could be used as a screening method to predict fumonisin concentration in a bulk lot of shelled corn.

Another study on sampling methodology was carried out by Casado et al. (2010). These authors focused on the analysis of the two- and three-dimensional spatial structure of FB_1 and FB_2 in maize in a bulk store using a geostatistical approach and how the results help to determine the number and location of incremental samples to be collected. For that purpose, samples were taken in three planar layers inclined at 45 degrees parallel to the open face of a maize bed with an area of 3800 m^2 and a height of 10 m. Each sample of 200 g was taken using a specially designed probe, which was inserted horizontally through the face, and analysed for the percentage of kernels infected by *Fusarium* and the presence of FB_1 and FB_2 by HPLC-MS. The results of the geostatistical analysis showed no evidence of spatial structure for fumonisins in two dimensions, which could be due to the nature of the mycotoxins, the way in which the grain was mixed and stored, the presence of spatial structure at a finer or coarser scale than the sampling grid or insufficient data points. The number of *Fusarium*-infected kernels was not a good indicator for the prediction of fumonisin concentration.

Miraglia et al. (2005) proposed a holistic view for an ideal sampling plan, which is based on two consecutive steps: 1) establish "why, where and when" sampling has to be performed and 2) establish "how" to draw samples by assessing practical ad hoc guidelines, considering that, for bulk goods in particular, mycotoxins are not all homogeneously distributed in a lot. They concluded that an effective plan to statistically evaluate the level and impact of mycotoxin contamination should consist of "statistically" based information on the sites where samples are to be collected and the reliable evaluation of the status of contamination at the sites.

Great efforts thus far have been devoted to improving the reliability of the analytical measurements. In item 8 of the agenda of the Fourth Session of the Codex Committee on Contaminants in Foods (FAO/WHO, 2010a,b), sampling plans for whole (shelled) maize, corn on the cob and maize products, such as maize flour, meal, grits and processed maize flour, were proposed. For maize products, it was assumed that the sampling variance for these commodities was similar to that associated with aflatoxin in comminuted feeds. However, recent data reviewed for this evaluation showed that the fumonisin distribution in foods and feeds is close to normal. The Committee concluded that further investigations of fumonisin distribution in different foods and feeds are necessary to improve the sampling protocols for fumonisins.

5. EFFECTS OF PROCESSING

Mycotoxin production can occur in the field and during harvest, processing, transport and storage of food and feed. Several factors, such as moisture content,

temperature and pH, among others, affect the production of mycotoxins. Apart from sorting to remove contaminated parts or units, levels of mycotoxins in contaminated commodities may be reduced or redistributed by food processing procedures (Dall'Asta et al., 2009b). It is therefore very important to know the stability and possible chemical changes of the different mycotoxins during food processing.

5.1 Sorting and cleaning

Sorting and cleaning may lower the fumonisin concentration by removal of the contaminated material, but they do not destroy the mycotoxins. However, in rural populations, where the corn used for human consumption can be of poor quality, sorting visibly diseased kernels from lots of corn is effective at reducing fumonisin levels (Jackson & Jablonski, 2004), and Afolabi et al. (2006) proposed it as a technique to reduce fumonisin levels by subsistence farmers.

Cleaning treatments, such as sieving fines or broken kernels from bulk shipments of corn, can reduce fumonisin levels by over 50% (Saunders, Meredith & Voss, 2001). Pietri, Zanetti & Bertuzzi (2009) found an average reduction in a cleaning step of 45% on two heavily contaminated maize lots. An overall mean free FB_1, FB_2 and FB_3 reduction of 71% was obtained by sorting out the visibly damaged kernels. Hand washing of the sorted good maize kernels for a period of 10 minutes at 25 °C resulted in optimal reduction of fumonisin (approximately 10%), according to Van der Westhuizen et al. (2011b).

D'Ovidio et al. (2007) studied the effects of the popcorn popping procedure using microwave heating for the destruction of FB_1 and FB_2. Cleaned commercial popcorn ready for packaging contains virtually no fumonisin, but the gravity sort reject stream contained approximately 5 mg/g, and the optical reject stream contained approximately 1 mg/g. Where fumonisins are present in popcorn, popping in the microwave oven resulted in a 67–92% reduction.

Fumonisin reduction could depend on the initial mycotoxin contamination, and further studies should be done to evaluate this.

5.2 Dry and wet milling

The milling process appears to result in the differential distribution of fumonisins in different milling fractions, leading to an increase in toxin concentrations in some products and a decrease in others (Kabak, 2008).

Dry milling is the physical process by which the components of the grain are separated, giving rise to the bran, the germ and other raw ingredients, such as corn grits, cornmeal and corn flour, that are used to manufacture breakfast cereals, snack foods and bakery products (Jackson & Jablonski, 2004). Fumonisins were not found to be destroyed during the dry milling process and were found in all fractions, with higher concentrations in bran and germ (Brera et al., 2004; Castells et al., 2008; Pietri, Zanetti & Bertuzzi, 2009; Vanara, Reyneri & Blandino, 2009). These results were in accordance with the ones found in a dry milling plant in Argentina by Broggi et al. (2002), where it was reported that dry milling of FB_1-, FB_2- and FB_3-contaminated corn tended to concentrate the fumonisins in the bran

and germ fractions (concentration 3 times the value of the initial corn fumonisin concentration) and produced grits relatively free of contamination (approximately 10% of the initial fumonisin corn concentration). Pietri, Zanetti & Bertuzzi (2009) found a similar reduction of FB_1 concentration in the corn grits produced in an Italian plant. Scudamore & Patel (2009) found that the general patterns of distribution of French and Argentine maize in three large commercial maize mills in the United Kingdom were similar but very variable, and they concluded that it depended on milling strategies as well as the maize consignment.

Wet milling of whole corn generally results in the production of fractions called starch, germ, gluten and fibre. Wet milling of contaminated corn produces a starch fraction with very little or no FB_1 and FB_2 (Saunders, Meredith & Voss, 2001; Resnik, Pacin & Funes, 2006). The median values found were 6140 µg/kg for FB_1 and 2170 µg/kg for FB_2 in whole corn; the obtained germ was contaminated with a median of 1211 and 1032 µg/kg, gluten meal with 2062 and 3317 µg/kg and gluten feed with 1020 and 224 µg/kg for FB_1 and FB_2, respectively. The reduction was due to the partial solubility of the fumonisins in the steep water.

5.3 Thermal processing

Thermal processing is used by the food industry to convert corn into a multitude of consumer products, such as muffins, breakfast cereals, snacks and breads. Fumonisins are relatively heat stable (up to 100–120 °C) and therefore survive many of the conditions used in cooking and food manufacturing, as described in the monograph from the fifty-sixth meeting of the Committee (Annex 1, reference *153*).

Some studies demonstrated that no significant removal of fumonisins occurs during processes that reach temperatures less than 125 °C (Jackson & Jablonski, 2004). Shephard et al. (2002b) found a moderate reduction of 23% of fumonisin when South African maize porridge was prepared by boiling salted cornmeal for 20 minutes in water. Brera et al. (2004) found that the cooking of polenta in a domestic pressure cooker did not affect the fumonisin B level of contamination. Similar results were also reported by Kpodo et al. (2006) in the preparation of kenkey (Ghanaian fermented maize product) in the boiling step of 3 hours, in which no fumonisin reduction was found. Processes such as baking and canning, where the product rarely reaches 175 °C, result in little or no loss of fumonisin (Bullerman & Bianchini, 2007).

In contrast, foods that reach temperatures above 150 °C (baked, extruded, fried) may have losses of fumonisins (Saunders, Meredith & Voss, 2001; Bullerman & Bianchini, 2007; Bullerman et al., 2008). Voss, Meredith & Bacon (2003) reported that pan-frying corncake at 218 °C for 10–12 minutes reduced the free FB_1 and FB_2 concentrations by an average of 29.2%. Baking muffins using cornmeal spiked with FB_1 resulted in significant losses of this mycotoxin (52–57%), as reported by Avantaggiato et al. (2003). The effectiveness of baking and frying for reducing fumonisin concentrations in food is somewhat variable and depends on product type and cooking conditions. Factors tending to reduce fumonisins are longer cooking times, higher cooking temperatures and higher moisture content of the dough.

Extrusion is a process by which cornmeal or corn grits are subjected to high pressures, temperatures and shear forces and is widely used in the production of breakfast cereals, snacks and textured food. It has been demonstrated that extrusion cooking can lead to a reduction of mycotoxin levels in different cereals (Castells et al., 2005). Bullerman et al. (2008) found that extrusion decreases FB_1 concentrations by 21–37%, whereas the same process with added glucose decreases FB_1 concentrations by 77–87%. The addition of sucrose and salt and salt alone also led to a significant decrease in FB_1 concentrations in extruded corn flour (Castelo et al., 2001; Scudamore et al., 2008; Voss et al., 2008; Castells et al., 2009). Corn flakes presented a reduction of FB_1 and FB_2 concentrations ranging from 60% to 70% during the entire extrusion process, according to De Girolamo, Solfrizzo & Visconti (2001b) and Scudamore & Patel (2008). The moisture content of the cereal feed during extrusion has a great effect on the loss of fumonisin, but this effect is not easy to explain (Scudamore et al., 2008). Scudamore, Scriven & Patel (2009) found that the amount of FB_1 + FB_2 in tortilla chips prepared from maize flour by extrusion, heating and frying was reduced by an average of 59%. Extrusion cooking generally decreases the mycotoxin levels at rates depending on factors such as the type of extruder, type of screw, die configuration, barrel temperature, screw speed, moisture content of raw material, use of additives and the initial mycotoxin concentration.

However, it is important to point out that in thermal processes, it is not yet very clear if the reduction of fumonisins is due only to thermal decomposition of the mycotoxins or also to binding to proteins, sugars or other compounds in the food matrix. Unlike other mycotoxins, fumonisins may undergo a common Maillard reaction with its primary amine group when heated in the presence of a reducing sugar (Ryu, Bianchini & Bullerman, 2008). Incubation of FB_1 and D-glucose in aqueous solution resulted in the formation of *N*-(1-deoxy-D-fructos-1-yl) FB_1 and *N*-(carboxymethyl) FB_1 (Poling, Plattner & Weisleder, 2002). Protected lysine and cysteine methyl esters reacted with FB_1, demonstrating that free thiol or amino groups of proteins are likely to react with fumonisins as well (Berthiller et al., 2009). In a starch model, FB_1 was able to react with methyl-α-D-glucopyranoside via tricarballylic acid side-chains to form FB_1-di-(methyl-α-D-glucopyranoside) (Humpf & Voss, 2004).

Further investigations are necessary to determine the fate of fumonisins and their reactions in heated food.

5.4 Alkaline treatment

Nixtamalization is a traditional process of cooking and steeping the corn with calcium hydroxide and heating to produce masa flour that will be used to make snacks and tortilla products. This process can reduce the fumonisin concentration by 50–80%, but when these alkaline conditions are applied, the hydrolysis of the tricarballylic esters at C-14 and C-15 gives rise to partially hydrolysed or totally hydrolysed fumonisin B (Burns et al., 2008). Cortez-Rocha et al. (2002) found that FB_1 was reduced by 82% during the nixtamalization step because it was transformed into its hydrolysis product, HFB_1. Palencia et al. (2003) found that tortillas prepared using the traditional nixtamalization method of Mayan communities contained FB_1,

FB_2 and FB_3 and their hydrolysed counterparts. There were equimolar amounts of FB_1 and HFB_1 in tortillas, but the sum of FB_1, FB_2 and FB_3 was reduced by 50%.

De la Campa, Miller & Hendricks (2004) showed that the amount of FB_1 detected in the masa and tortillas decreased as the concentration of calcium hydroxide increased in the process of nixtamalization. They found that the higher the initial concentration of FB_1, the greater the reduction of FB_1.

Apart from nixtamalization, Lefyedi & Taylor (2006) studied the effect of alkaline steeping on the presence of mycotoxins in sorghum malt. These authors found no detectable amounts of fumonisin after a treatment with 0.2% sodium hydroxide.

These hydrolysed forms could also be involved in binding with different compounds of the food matrix, so this should be taken into account in the development of future extraction methods.

5.5 Fermentation

Ethanol fermentation by yeast has little or no direct impact on the level of mycotoxins, according to Ryu, Bianchini & Bullerman (2008). In general, mycotoxins tend to concentrate in by-products of the ethanol fermentation process, such as dried distillers' grains and dried distillers' grains with solubles, which are likely to be included in feed (Wu & Munkvold, 2008) in ruminant and non-ruminant animal diets (Busman & Kendra, 2008).

The fate of fumonisin in traditional fermented foods in Africa was studied by Fandohan et al. (2005). Mawe, ogi and lifin are traditional African maize-based foods, prepared after sorting, winnowing, washing and crushing maize grains. Makune and akassa are fermented mawe and ogi, respectively. Although the fermentation of mawe into makune did not present any reduction of fumonisin, fermented ogi (akassa) showed a 13% reduction. Kpodo et al. (2006) found, in the preparation of kenkey (Ghanaian fermented maize product), that a 3-day fermentation had no significant effect on fumonisin levels.

In contrast, microbial fermentation was studied by Siruguri, Ganguly & Bhat (2009), who explored the used of spontaneous fermentation of mouldy sorghum seeds intended for feed. Fermentation of mouldy sorghum decreased FB_1 concentrations to non-detectable levels at 24 and 36 hours.

Mokoena, Chelule & Gqaleni (2005) showed a 75% reduction of FB_1 levels in maize meal after fermentation with lactic acid bacteria for 4 days, although the safety of the decontaminated maize-based food is not guaranteed because of the possible reversibility of the lactic acid bacteria binding under certain conditions. Chelule et al. (2010) studied the effect of lactic acid fermentation of amahewu (traditional South African maize-based porridge) on FB_1. Increased levels of protein were observed in amahewu, especially in the samples with added yeast and bread flour, in comparison with the levels in starter maize. An 87% reduction of FB_1 was found in amahewu, but it is not clear if this reduction is due to the fermentation process per se or due to the lactic acid bacteria binding to mycotoxins. As the amount of protein found is also quite high, FB_1 could also be bound to the proteins. Additional studies on the use of microorganisms to reduce fumonisin levels are discussed in section 6.2.

5.6 The issue of hidden and bound fumonisins

Fumonisins are relatively heat stable and are appreciably degraded only at higher temperatures, such as those connected to baking, roasting or extrusion, or, alternatively, by alkali processing (Dall'Asta et al., 2008). It is also well known that processing decreases fumonisin concentrations, probably by changing their chemical structures (Seefelder, Knecht & Humpf, 2003). The first fumonisin degradation products described in the literature were the hydrolysed fumonisins (HFB_x) (Humpf & Voss, 2004). These hydrolysed fumonisins are formed during alkali treatment (e.g. nixtamalization) in processed foods, as was previously described.

Apart from this, several publications have demonstrated the presence of fumonisins potentially bound to or strongly associated with proteins or other food components, which escape conventional analysis (Dall'Asta et al., 2010), as was described in section 3.1.

In a study carried out on several commercial maize-based products for total and bound fumonisins, it was found that in corn flakes, the amount of HFB_1 released after hydrolysis (bound fumonisin) is 4 times higher than the sum of FB_1 and HFB_1 (Dall'Asta et al., 2008). Interestingly, bound forms were also found to occur not only in thermally treated products (e.g. corn flakes), but also in mild processed foods (e.g. cakes) and even maize flour (Kim, Scott & Lau, 2003; Seefelder, Knecht & Humpf, 2003; Park et al., 2004). These could be due to the transformation of native fumonisins to bound derivatives by the natural phenomena of plant metabolism (Dall'Asta et al., 2008).

In addition to hydrolysed fumonisins, there exist other derivatives that could be formed during the processing of corn. Fumonisins are able to react with reducing sugars in a manner similar to non-enzymatic browning and also with starch. The primary amine group of fumonisins reacts with the carbonyl group of reducing sugars, forming Schiff base, which converts into further products. Seefelder, Knecht & Humpf (2003) incubated FB_1 and HFB_1 with β-D-glucose and sucrose (as a model for reducing sugars) and with methyl β-D-glucopyranoside (as a model for starch). The incubation with the reducing sugars resulted in the formation of Amadori rearrangement product conjugates, and the model proposed for starch resulted in a diester of the fumonisin tricarballylic acid side-chains with methyl β-D-glucopyranoside. Other studies have focused on the protein-bound fraction. Covalent binding between fumonisins and protein fractions was also reported in maize flour (Dall'Asta et al., 2008) and corn flakes (Kim, Scott & Lau, 2003).

As was stated previously, the issue of hidden fumonisins (e.g. binding and hiding phenomena) requires further investigation in order to get a more complete understanding of the fumonisin issue in corn-based products.

6. PREVENTION AND CONTROL

Strategies to reduce fumonisins may be grouped under pre-harvest or post-harvest interventions. Pre-harvest interventions include cultivar practices and crop management, host plant resistance through breeding and/or genetic engineering and biocontrol (Munkvold, 2003). Post-harvest interventions include

improved drying and storage conditions and the use of physical, chemical and microbiological interventions. The most effective and economical way to keep mycotoxin contamination under control, however, is the development and use of resistant hybrids (Cavaliere et al., 2007).

6.1 Pre-harvest control

Conventional breeding and selection methods have been used for centuries to improve maize resistance to fungal and insect infection and can be considered as the best solution to *Fusarium* control in susceptible crops. Lines have been produced that provide good resistance to *Fusarium* sp. (Jouany, 2007). Transgenic approaches are also being developed as an additional strategy aimed at either reducing mycotoxin biosynthesis or detoxifying mycotoxins in the crops themselves. Such approaches could provide further protection for the grower in environments in which fumonisins present a risk to the crop, even when the maize is relatively resistant to *Fusarium* infection (Duvick, 2001). Insect damage predisposes maize to fumonisin contamination by facilitating fungal infection. Transgenic Bt maize contains a gene from the soil bacterium, *Bacillus thuringiensis*, that produces proteins toxic to key insect pests of maize. Depending on the severity of insect infestation in a given year, fumonisin reductions afforded by Bt maize can greatly increase the amount of crop acceptable for human consumption (Munkvold, Hellmich & Showers, 1999; Hammond et al., 2004; de la Campa et al., 2005).

Field management practices effective for reducing the incidence of mycotoxin contamination in the field have been reviewed by Bruns (2003) and include timely planting, proper plant nutrition, avoidance of drought stress, particularly during kernel filling, controlling certain insect pests and proper harvesting.

In recent years, the use of antagonistic microorganisms to control undesirable moulds and reduce fumonisin levels has received attention (Pereira et al., 2007; Gwiazdowska et al., 2008; Kabak & Dobson, 2009). The treatment of seeds with *Bacillus amyloliquefaciens* and *Microbacterium oleovorans* reduced *Fusarium verticillioides* counts in maize grains and significantly decreased the amounts of FB_1 and FB_2 in grains from plants grown from the treated seeds (Pereira et al., 2007). A *Propionibacterium* culture and a cell-free supernatant fraction from the culture also decreased fumonisin production by *F. verticillioides* by 90% and 88.4%, respectively (Gwiazdowska et al., 2008). Additional studies are, however, required to characterize the antifungal and antimycotoxigenic mechanisms involved.

Laboratory studies on the effect of naturally occurring phenolic compounds on growth of *Fusarium verticillioides* and the reduction of FB_1 production showed that chlorophorin, iroko, maakianin, vanillic acid and caffeic acid inhibited mould growth and reduced FB_1 production by between 90% and 94%, with chlorophorin being most effective (Beekrum et al., 2003).

6.2 Post-harvest control

Fusarium species are predominantly considered to be field fungi, but it has been reported that fumonisin production can occur post-harvest when storage conditions are inadequate (Marin et al., 2004). Factors to consider have been

reviewed by Jouany (2007) and include the physiological stage of the plant at harvest, humidity level before and during storage as well as the temperature during storage.

As a result of the multiple possible origins of fungal infection, any prevention strategy for fungal and mycotoxin contamination must be carried out at an integrative level all along the production chain. The intervention steps proposed (Jouany, 2007) are before fungal infestation, during fungal invasion of plant material and mycotoxin production and finally when products have been identified as contaminated. Fumonisins fit into a hazard analysis and critical control point (HACCP) programme, and appropriate critical control points and their critical limits must be identified. Key management aspects to consider after harvest include efficient and prompt drying of maize or cereals for medium- and long-term storage in hygienic silos free of insect pests and fungal populations; and accurate and regular moisture content measurement to ensure that safe thresholds are not breached (Chulze, 2010). The *FAO/IAEA Manual on the Application of HACCP System in Mycotoxin Prevention and Control* (FAO/IAEA, 2001) is available and provides examples in which the HACCP system is applied to mycotoxin contamination in food and feed, especially in developing country scenarios.

Widespread public concern about the long-term health and environmental effects of synthetic pesticides has resulted in the evaluation of natural pesticides of both microbial and plant origin to control fungal spoilage and mycotoxin production in stored grains (Chulze, 2010). Several studies using antioxidants, essential oils under different conditions of water activity, and temperature and controlled atmospheres have been evaluated as possible strategies for the reduction of fungal growth and fumonisins in stored maize (Velluti et al., 2003; López et al., 2004; Velluti, Sanchis & Ramos, 2004; Samapundo et al., 2007; Chulze, 2010; Dambolena et al., 2010). These treatments are associated with high costs, especially for large-scale use (Chulze, 2010). Various studies (López et al., 2004; Velluti, Sanchis & Ramos, 2004) have reported that some essential oils cause reduction in fumonisin production by *F. verticillioides*, with some of the antitoxigenic properties of these oils being attributed to monoterpenoids (Lambert et al., 2001). A recent study with menthol showed stereospecific antifungal activity and enantiospecific antifumonisin activity of the menthol stereoisomers (Dambolena et al., 2010). This study highlights the importance of taking the chirality of the compound into account when studying the antifungal and antimycotoxigenic mechanisms of the monoterpenes. An aroma constituent of many fruits and vegetables, the aldehyde *trans*-2-hexenal, when used for post-harvest fumigation, inhibited *F. verticillioides* growth. Its efficacy was dependent on the concentration and time of incubation. The most effective dose was 369 µl/l, but with off-odours in maize kernels. In contrast, fumonisin levels were not reduced but rather were stimulated at high (369 µl/l) doses. The phenols (carvacrol, main constituent of oregano and thyme essential oils, and eugenol, constituent of cinnamon and clove essential oils) also showed fungicidal activity against *F. verticillioides* in in vitro trials (Menniti, Gregori & Neri, 2010). These are all recent developments, and their commercial or large-scale applicability remains to be seen.

Since the monograph was written for the fifty-sixth meeting of the Committee, additional studies conducted with gamma-irradiation showed decreased viable

counts of *Fusarium* in seeds with increasing radiation dose levels. Growth of *Fusarium* spp. was inhibited at 4.0 kGy for barley and 6.0 kGy for wheat and maize. Additionally, application of a radiation dose at 5 kGy inactivated FB_1 by 96.6%, 87.1% and 100% for wheat, maize and barley, respectively, whereas a dose of 7 kGy was sufficient for complete destruction of FB_1 in wheat and maize (Aziz et al., 2007).

In general, mycotoxins are stable compounds, and those commonly found in cereal grains are not destroyed during most food processing operations, resulting in contamination or carry-over into finished cereal-based foods. Food processes that may have an effect on mycotoxins have been reviewed in section 5.

Physical separation of grain, irradiation and the use of ammoniation under varying conditions of pressure, temperature, time and moisture content as a means of reducing fumonisin levels were reviewed by the fifty-sixth meeting of the Committee. Since the last review, newly available information has confirmed reductions in fumonisin levels through cleaning, redistribution of fumonisins, dissolution of fumonisins in wash water and steep water and extrusion cooking (Brera et al., 2004; Fandohan et al., 2005; Kpodo et al., 2006; Bullerman & Bianchini, 2007; Van der Westhuizen et al., 2010a,b, 2011b). Detailed descriptions are given in section 5.

Another approach that has been studied for the reduction of fumonisins in grains is the use of microorganisms to either bind or degrade fumonisins. Twenty-nine strains of lactic acid bacteria and propionic acid bacteria tested for their ability to bind FB_1 and FB_2 showed positive results under acidic conditions. Binding was, however, decreased to nearly 0% at neutral pH. Up to 82% of FB_1 and up to 100% of FB_2 were removed at the bacterial concentrations used. Non-viable bacteria showed higher binding than viable bacteria, suggesting that changes in the cell wall resulting from heat inactivation treatment favourably modified and/or increased the availability of binding sites (Niderkorn, Boudra & Morgavi, 2006). Additional studies (Niderkorn et al., 2007) conducted under conditions simulating corn silage showed strains from *Streptococcus* (*Streptococcus thermophilus*) and *Enterococcus* capable of binding up to 24% and 62% of FB_1 and FB_2, respectively. Additional studies are given in section 5.5.

Regarding degradation, two fungal species, *Exophiala spinifera* (a "black yeast") and *Rhinocladiella atrovirens*, have been isolated from field-grown maize kernels in an effort to identify a biological means to facilitate the detoxification of fumonisins. These organisms possess the ability to use FB_1 as a sole carbon source and thus are a potential source of fumonisin detoxifying enzymes. The genes encoding the deesterification and oxidative deamination enzymes that degrade fumonisins have been cloned and expressed in transgenic maize (Duvick, Rood & Wang, 1998; Duvick, 2001).

A recently isolated bacterium from soil samples belonging to the *Delftia-Comamonas* group completely degraded FB_1 after incubation at 25 °C for 1 day, even when the mycotoxin was present as the sole carbon source (Benedetti et al., 2006). Microbiota from healthy pigs was also found to degrade FB_1 to HFB_1 by 46% within 48 hours in buffered solution (Fodor et al., 2007).

Progress has been made towards a process for the enzymatic detoxification of fumonisins in oxygen-limited conditions, such as in ensilaged forage or in the intestinal tract of animals. A bacterium designated *Sphingomonas* sp. MTA144 showing strong fumonisin-degrading activity was isolated by Täubel (2005). This organism was earlier referred to as *Sphingopyxis* sp. MTA144 (Takeuchi, Hamana & Hiraishi, 2001). Consequently, studies showed that gene products of the two genes of *Sphingopyxis* sp. MTA144, *fumD* and *fuml*, degrade through their consecutive action on the mycotoxin FB_1. The mechanism involved hydrolysis of FB_1 to HFB_1 by carboxylesterase with the loss of the two tricarballylic side-chains, followed by deamination of HFB_1 by aminotransferase in the presence of pyruvate and pyridoxal phosphate (Heinl et al., 2010).

7. LEVELS AND PATTERNS OF CONTAMINATION IN FOOD COMMODITIES AND FEED

Information on the natural occurrence of fumonisins was drawn from data received from a number of countries (Argentina, Australia, Brazil, Canada, China, Ghana, Japan, Republic of Korea, Singapore, United Republic of Tanzania, Uruguay, USA), results submitted by the European Food Safety Authority (EFSA) (Austria, Belgium, Cyprus, Czech Republic, Estonia, France, Germany, Hungary, Lithuania, Luxembourg, the Netherlands, Slovakia and Spain) and results submitted by Dr E.M. Binder, ERBER AG, Technopark 1, 3430 Tulln, Austria, on fumonisins in feeds from around the world, as well as surveys published in the open literature. The period of publication for the incorporation of data was 2001 to March 2011. Data collected from 59 countries have been tabulated by region and country in the fumonisin occurrence and co-occurrence tables in Appendix 1.

Data collected in the tables include information on LOD (or LOQ or both), number of samples analysed and percentage of negative samples. The mean values reported are the mean of all samples, with concentrations in samples below the LOD being taken as zero or a value of LOD/2 for the samples without detected contamination; this is noted in the occurrence table footnote. The maximum level analysed in a given set of samples is recorded, and in most cases the 90th percentile level was calculated.

From all the data analysed (28 567 samples), 6.1% were from Africa, 29.2% from the Americas, 22.9% from Asia, 41.0% from Europe and 0.8% from Oceania. The collected information included fumonisin levels in another 3769 samples of feed that arrived too late for incorporation in the occurrence tables. The evaluated data were on both foods (61.9%) and feeds (38.1%). Some works studied the presence of fumonisin and/or other mycotoxins in corn under the effect of different conditions, such as temperature, rainfall, planting date, levels of fertility and type of hybrid, so the analyses were carried out in field experiments, and the data were not included in the occurrence tables (Abbas et al., 2002, 2006; Abbas, Shier & Cartwright, 2004, 2007; Bruns & Abbas, 2005; Parsons & Munkvold, 2010). From all the co-occurrence data analysed (9714 samples), 5.4% corresponded to Africa, 31.2% to the Americas, 27.9% to Asia, 33.4% to Europe and 2.1% to Oceania.

Most of the products with fumonisin contamination are corn and corn-based products. Other products analysed for fumonisin contamination included wheat and wheat products, oat grain, sorghum, rice, soya, barley, asparagus, coffee, dried fruits, peanuts, beans, cassava chips, alcoholic beverages, potato, products for special nutritional use, vegetable-based meals, meals for infants, ices and desserts, sunflower seeds, vegetable oils, cocoa and herbal tea. Data submitted by Singapore showed several analysed commodities and food (data not shown in the occurrence tables), but FB_1 was detected in only a few samples (turmori powder, ground pepper, tempeh), and levels were less than 500 µg/kg.

In addition, data on hidden and bound fumonisins were collected. The occurrence table for hidden and bound fumonisins is also included in Appendix 1. This table indicates how the fumonisins were measured in the food item. The results are expressed sometimes as HFB_1 and sometimes as the equivalent of FB_1 (conversion coefficient 1.78).

Few reports have dealt with fumonisin carry-over to animal products. Four samples of meat with no detected fumonisin are reported in the occurrence tables. Gazzotti et al. (2009) analysed 10 commercial samples of milk and found that 8 contained FB_1 at a mean concentration of 0.26 µg/kg (range of positive samples 0.26–0.43 µg/kg). Another work carried out by Fink-Gremmels (2008) studied the bioconversion and transfer of FB_1 from feed to milk and proposed an estimated carry-over rate between 0% and 0.05%. EFSA concluded in 2005 that "Available data on carry-over of fumonisins from animal feeds into edible tissues, including milk and eggs, indicate that transfer is limited, and thus residues in animal tissues contribute insignificantly to total human exposure" (EFSA, 2005).

From the data analysed for food, most of the information corresponds to FB_1 or FB_2 or the sum of FB_1 + FB_2. Of the total corn samples analysed, only 475 included information on FB_1, FB_2 and FB_3 values. To study the percentage of each of these toxins in relation to the sum of the three, an exploratory analysis was performed. Figure 4 shows the box plot of these relationships.

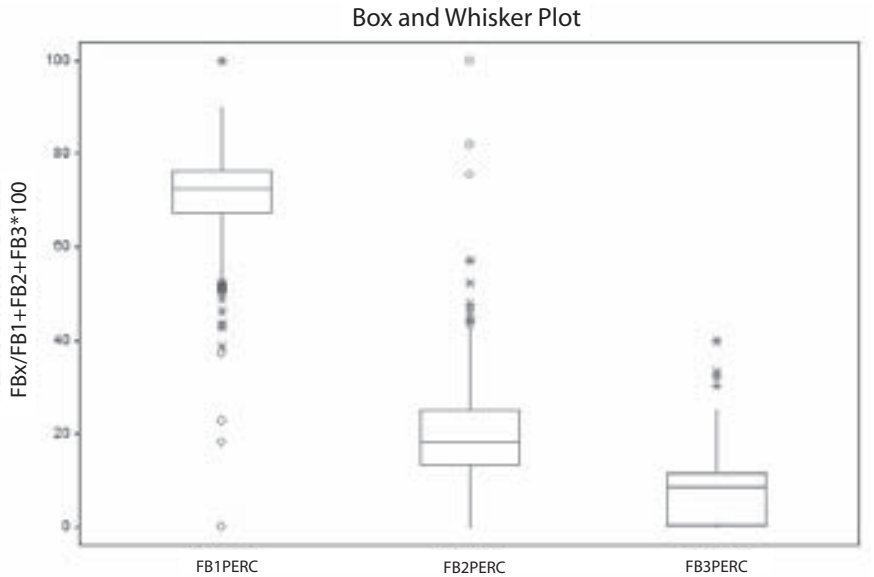
The mean values obtained for FB1PERC, FB2PERC and FB3PERC (as defined in Figure 4) are 72.7%, 19.3% and 8%, respectively. As can be seen in Figure 4, there are a lot of outliers. Therefore, it was decided to analyse each country's results separately. Table 10 shows the results divided by country.

This variation between FB1PERC and FB2PERC was also pointed out by de la Campa et al. (2005) in a study on fumonisin accumulation in maize grain within contrasting environments in Argentina and the Philippines between 2000 and 2002. The relationship of FB_1 to FB_2 was correlated with the *Fusarium proliferatum* prevalence.

Fumonisin ratios in food depend on the process to which they are subjected, sometimes resulting in different ratios between the free fumonisins in corn. As an example, Table 11 illustrates the relationship between the fumonisins for three different corn-based foods.

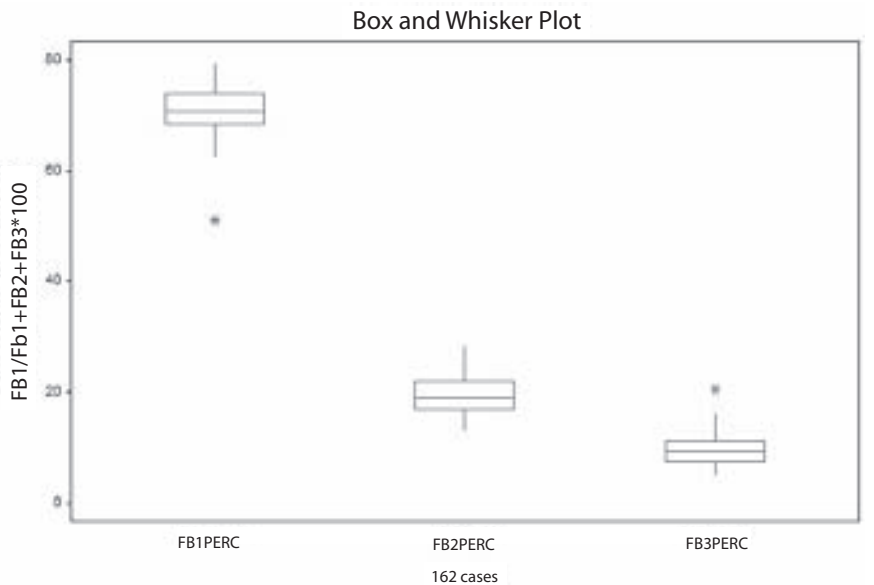
In contrast, the distribution of fumonisin in food tends to be almost normal. Figure 5 shows the box plot corresponding to the fumonisin distribution in fine bakery wares and salty biscuits in Germany.

Figure 4. Box plot of the FB_1, FB_2 and FB_3 relationships (%) in 475 corn samples



FB1PERC = $FB_1/(FB_1 + FB_2 + FB_3)\cdot 100$; FB2PERC = $FB_2/(FB_1 + FB_2 + FB_3)\cdot 100$; FB3PERC = $FB_3/(FB_1 + FB_2 + FB_3)\cdot 100$

Figure 5. Box plot of FB1PERC, FB2PERC and FB3PERC (%) in bakery wares and salty biscuits in Germany



FB1PERC = $FB_1/(FB_1 + FB_2 + FB_3)\cdot 100$; FB2PERC = $FB_2/(FB_1 + FB_2 + FB_3)\cdot 100$; FB3PERC = $FB_3/(FB_1 + FB_2 + FB_3)\cdot 100$

Table 10. Data obtained for FB1PERC, FB2PERC and FB3PERC for foods by country

Country	Number	Mean (%)	Minimum (%)	Median (%)	Maximum (%)
FB1PERC					
Argentina	179	71.1	37.1	70.4	100
China	184	73.5	0	74.3	100
Germany	16	71.5	61.2	71	81.8
Ghana	68	77.2	53.8	72.1	100
Japan	28	67.3	51.9	67.9	75.6
FB2PERC					
Argentina	179	23.7	0	25.1	47.9
China	184	15.5	0	14.4	100
Germany	16	17.1	11.4	16.4	27.1
Ghana	68	16.9	0	18.3	46.1
Japan	28	23.7	18.6	21.3	41.5
FB3PERC					
Argentina	179	5.2	0	5.7	33.4
China	184	11.1	0	11.2	40
Germany	16	11.4	4.6	11.6	20.9
Ghana	68	5.9	0	6.9	16.8
Japan	28	9.0	5.2	8.4	12.6

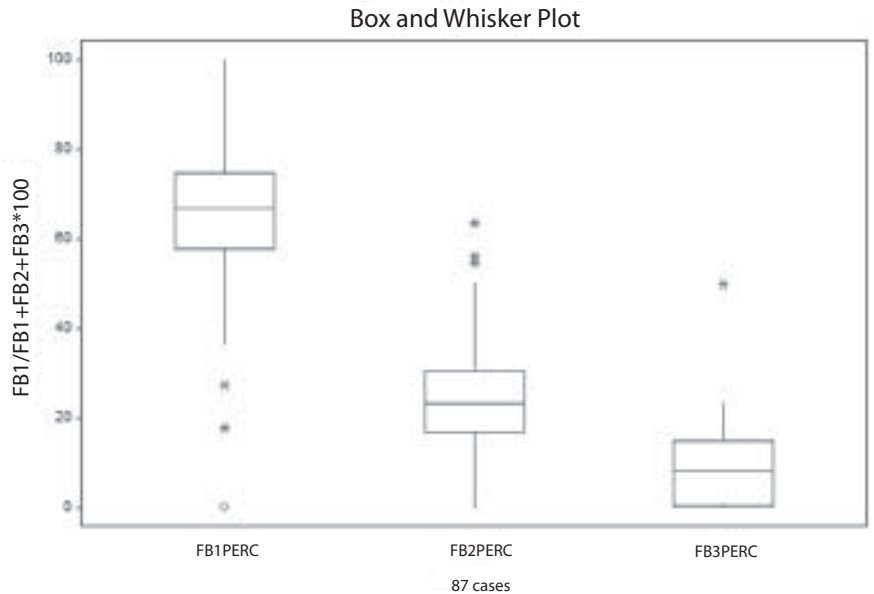
Table 11. Data obtained for FB1PERC, FB2PERC and FB3PERC in food

Country	Food	*N*	FB1PERC (%)	FB2PERC (%)	FB3PERC (%)
Ghana	Cooked fermented maize (kenkey)	55	90.3	8	1.7
Brazil[a]	Cooked flour	42	63.6	36.4	—
Germany	Fine bakery wares, biscuits, salty	54	70.8	19.4	9.6

[a] $FB_1 + FB_2$.

As is shown in the occurrence tables (Appendix 1), FB_2 can be present alone without FB_1 and FB_3 in *Coffea arabica* and canephora beans in Thailand, in one sample of red wine in Italy and in raisins sampled in the market in Denmark, which came from the USA, Chile and Turkey. Mogensen et al. (2010) showed the production of FB_2 and FB_4 on grapes and raisins. Regarding the presence of FB_2 in these products, this contamination may be due to *Aspergillus niger*. *Aspergillus*

Figure 6. Box plot of FB1PERC, FB2PERC and FB3PERC (%) in feeds from Japan



FB1PERC = $FB_1/(FB_1 + FB_2 + FB_3)\cdot100$; FB2PERC = $FB_2/(FB_1 + FB_2 + FB_3)\cdot100$; FB3PERC = $FB_3/(FB_1 + FB_2 + FB_3)\cdot100$

niger is also known to produce FB_4. Knudsen et al. (2011) analysed the presence of these fumonisins in samples of raisins bought in the market in Denmark. These authors found that one of the samples from Turkey and five from the USA were positive for FB_4. This information suggests that the presence of *Aspergillus niger* should be considered for further evaluation.

Ghiasian et al. (2006) studied the presence of the analogue 3-*epi*-FB_3 in samples of corn in the Islamic Republic of Iran. Overall, 10 samples of corn were found to contain the minor fumonisin analogue 3-*epi*-FB_3, although the maximum level found was 191 µg/kg, determined in one sample from Mazandaran Province. It accounted on average for only 1.8% (maximum 2.8%) of the total fumonisins (FB_1 + FB_2 + FB_3) in these samples (3-*epi*-FB_3 LOD <10 µg/kg). This was the only work found in the literature that analysed the presence of 3-*epi*-FB_3.

From the data analysed for feed, most of the information corresponds to FB_1 and/or FB_2 or the sum of FB_1 + FB_2. Figure 6 shows the relationship variations between the three fumonisins in feed from samples in Japan. The mean values obtained for FB1PERC, FB2PERC and FB3PERC were 65.8%, 25.5% and 8.8%, respectively, assuming that values lower than the LOD are equal to 0.

The analysis of 7085 (3116 included in the occurrence tables) individual data all over the world for feeds submitted by Dr E.M. Binder shows that the relationship between FB_1 and FB_2 cannot be represented by a single percentage. Table 12

Table 12. Data obtained for FB1PERC and FB2PERC for feeds by country

Country	Number	Mean (%)	Median (%)	Mean (µg/kg)	Maximum (µg/kg)
FB1PERC					
Africa	200	72.9	71.5	485.1	7 310
Europe	112	79.8	80.4	759.2	10 845
USA	61	77.8	79.3	774.4	3 390
FB2PERC					
Africa	196	20.2	28.5	185.8	3 175
Europe	111	15.5	19.6	278.5	5 094
USA	50	22.1	20.6	273.1	1 142

shows FB1PERC and FB2PERC calculated from individual data submitted from Africa, Europe and the USA from HPLC quantification.

There are different distributions of free fumonisin contamination in feeds by region (data not shown). The data submitted to the Committee by Japan were analysed in more detail. Figure 7 shows the box plot of the transformed data for FB_1, FB_2, FB_3 and total fumonisins (the transformation applied was ln(x + 1), to avoid indefinites).

The Kolmogorov-Smirnov goodness-of-fit test was applied to the data. The log-normal distribution provides an adequate fit for FB_1 and total fumonisins (*P*-values: 0.516 and 0.4275, respectively).

On Figure 8, a QQplot of log(FB_1 + 1) versus normal distribution is presented.

In the case of bound and hidden fumonisins, work conducted by Kim, Scott & Lau (2003) in corn flakes showed that the amount of protein-bound fumonisin was higher than the free FB_1 detected.

The distribution of bound and hidden fumonisins for three products, gluten-free snacks, gluten-free pasta/bread/flour and gluten-free corn flakes, is shown in Figure 9. The mean values for EXTRACTPE and HIDDENPER (as defined in Figure 9) are 63.8% and 37.2%, respectively.

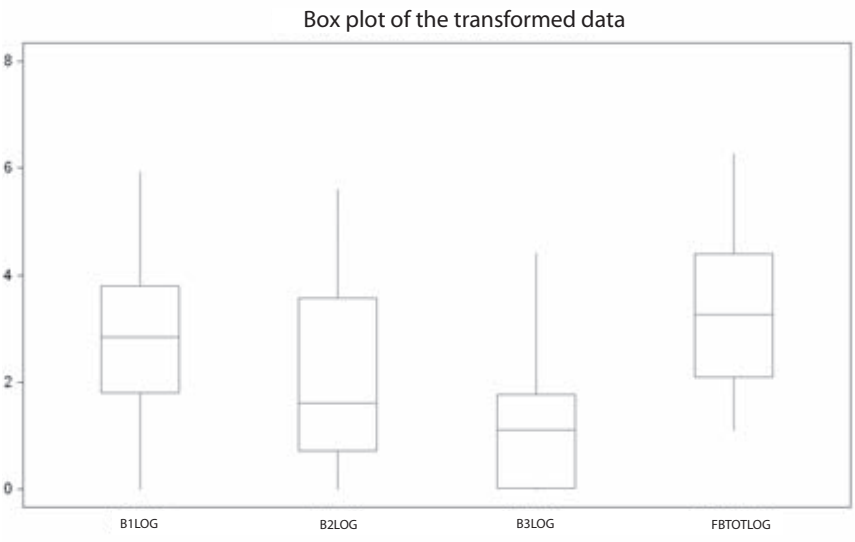
Despite these data, few works have focused on the issue of hidden and bound fumonisins, so further studies are needed to obtain more information about these fumonisins.

8. FOOD CONSUMPTION AND DIETARY EXPOSURE ASSESSMENT

8.1 Concentration in foods

For this assessment, concentrations of fumonisins in food commodities and in some processed foods were reported to FAO/WHO or obtained from the

Figure 7. Box plot of the transformed data for FB_1, FB_2 and FB_3 and total fumonisins (FB_T) in feeds from Japan



B1LOG = ln(FB_1 + 1), B2LOG = ln(FB_2 + 1), B3LOG = ln(FB_3 + 1), FBTOTLOG = ln(FB_T + 1)

Figure 8. QQplot of the transformed FB_1 (log(FB_1 + 1)) for feeds in Japan

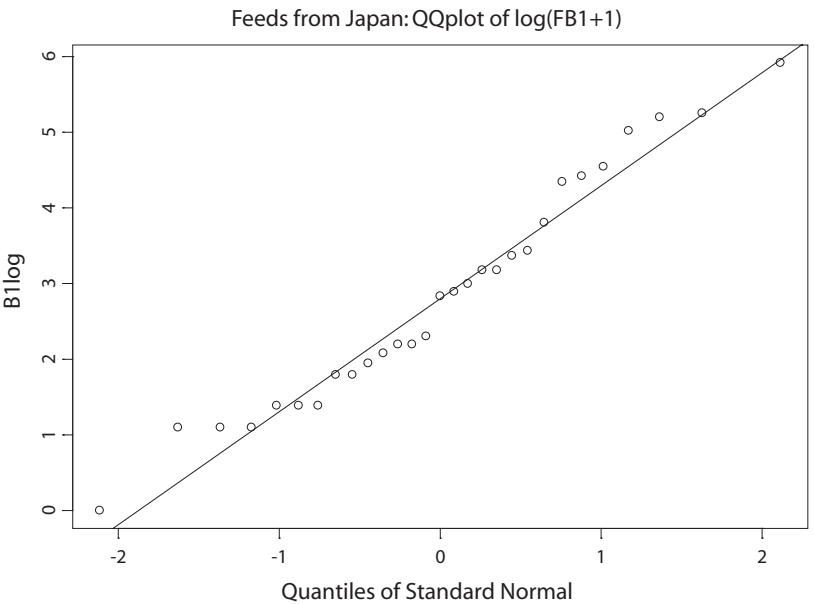
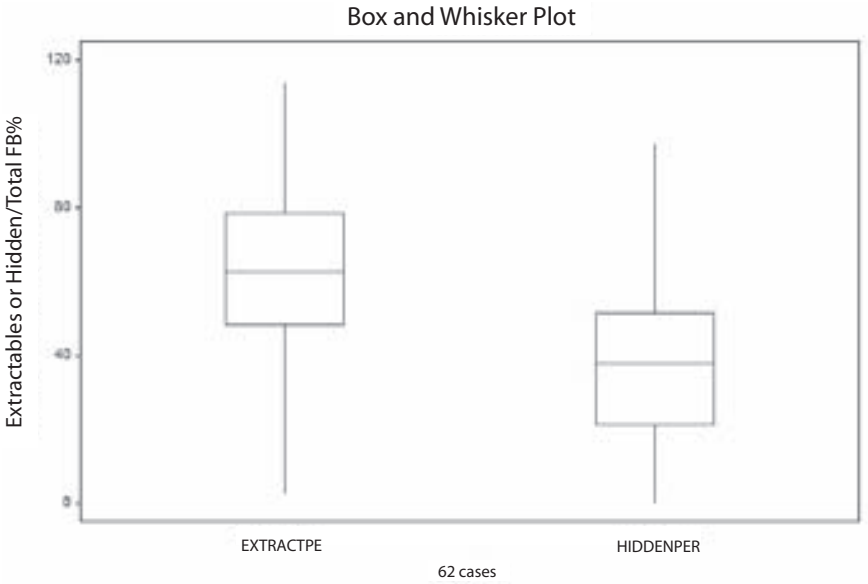


Figure 9. Box plot of the extractable and total fumonisins in gluten-free products in Italy (calculated from Dall'Asta et al., 2010)



EXTRACTPE = extractable fumonisins/total fumonisins·100; HIDDENPER = hidden fumonisins/total fumonisins·100

literature. The quality and reporting of the data are discussed in the previous section. Where possible, occurrence data were classified according to the food groups used in the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diets (Annex 1, reference *176*), which include information on consumption of raw or minimally processed foods. Data originating in 47 countries were analysed in this section, representing the 13 GEMS/Food consumption cluster diets.

Occurrence levels of fumonisins from processed and not sufficiently described foods were categorized separately and not used to estimate chronic dietary exposure. This is the case, for example, for analytical results reported for "mixed cereals", "breakfast cereals", "cereal-based dishes", "biscuits", "organic cookies", etc. The large majority of the available occurrence data on fumonisins refer to maize (harvested maize, corn flour, corn flakes, corn grit, corn starch, etc.) and "sweet corn, kernels" (canned and frozen corn, sweet corns, corn kernels and popcorn). In addition to these, chronic dietary exposure was assessed using information on other cereals (wheat, sorghum, rice, barley, millet, oats and buckwheat), "soya bean (dry)", "groundnuts, shelled" and "figs, dried". Occurrence data on "cereal-based baby and infant foods" were also reported, but infant exposure estimates were not assessed, as no consumption data on infants are available in the GEMS/Food consumption cluster diets.

Two scenarios were considered when calculating the mean values: samples in which the concentration was below the LOQ or LOD were assumed to have a

value of either zero (lower-bound scenario) or the limit itself (upper-bound scenario). In a few cases related to wheat (15 data points) and maize (3 data points), where the mean occurrence among positive samples was not available, the median or the maximum value was used to represent values above the LOD or LOQ.

When not reported, the concentration of total fumonisins was estimated by summing the concentration of FB_1, FB_2 and, when available, FB_3. Only in the case of "maize" and "sweet corn, kernels" was total fumonisin concentration estimated from FB_1 only by assuming that its occurrence represents 73% of the total. This procedure allowed the addition of 5638 samples to the 6985 for which total fumonisins was reported. The majority of the added samples were estimated from FB_1 only (42%) and from the sum of FB_1, FB_2 and FB_3 (32%); only 20% of the estimated total fumonisins resulted from the sum of FB_1 and FB_2 only.

The concentration data for FB_1 and total fumonisins for the consumption cluster diets are shown in Tables 13 and 14.

8.1.1 Fumonisin B_1

In total, data on 10 354 samples analysed for FB_1 were considered in the exposure assessment (43% from the Americas, 34% from Asia, 12% from Africa, 10% from Europe and 0.1% from Oceania). The large majority of the excluded samples (5058 samples) referred to unspecified cereals and cereal-based products and unspecified breakfast cereals, 2414 and 793 samples, respectively.

Information was available on FB_1 concentrations in maize from 12 GEMS/Food consumption cluster diets (cluster F is the only exception), in "sweet corn, kernels" from 5, in wheat, "soya bean (dry)" and rice from 3, in barley and sorghum from 2 and in all other foods from only 1. More than three quarters of the samples considered in the assessment of exposure (83%) referred to maize, followed by "sweet corn, kernels" (7%). It was noted that FB_1 is a common contaminant in maize and maize-based products, but levels vary widely between and within regions (Table 13). The average FB_1 concentration in maize ranged, across the different clusters, from 84 to 4322 µg/kg and from 93 to 4323 µg/kg under the lower- and upper-bound scenarios, respectively. Relatively lower levels were detected in "sweet corn, kernels" in cluster K (389–397 µg/kg, lower- and upper-bound means), in "figs, dried" in cluster B (238–250 µg/kg), in wheat in cluster E (221–221 µg/kg) and in barley in cluster M (212–212 µg/kg). Upper-bound mean levels of FB_1 in all other commodities and clusters did not exceed 200 µg/kg.

8.1.2 Total fumonisins

In total, occurrence levels of total fumonisins for 12 392 food samples (49% from the Americas, 27% from Asia, 13% from Europe, 11% from Africa and 0.1% from Oceania) were used to assess dietary exposure. The large majority of the excluded samples (2680 out of 4674 samples) referred to unspecified cereals and cereal-based products.

Information was available on total fumonisin concentrations in maize from 12 GEMS/Food consumption cluster diets (cluster F was the only exception), in "sweet corn, kernels" from 6, in barley from 4, in wheat and rice from 3, in buckwheat,

Table 13. Summary of data on concentrations of fumonisin B_1 in commodities from the GEMS/Food clusters

Group	Statistic[a]	Global total[b]	GEMS/Food clusters												
			A	B	C	D	E	F	G	H	I	J	K	L	M
Barley	No. of individual samples	175	—	—	—	—	—	—	—	—	—	—	—	146	29
	% sample < LOD or LOQ	82.3	—	—	—	—	—	—	—	—	—	—	—	98.7	0.0
	Mean (lower bound; µg/kg)	35.2	—	—	—	—	—	—	—	—	—	—	—	0.0	212.1
	Mean (upper bound; µg/kg)	43.7	—	—	—	—	—	—	—	—	—	—	—	10.3	212.1
Buckwheat	No. of individual samples	95	—	—	—	—	—	—	—	—	—	—	—	95	—
	% sample < LOD or LOQ	100.0	—	—	—	—	—	—	—	—	—	—	—	100.0	—
	Mean (lower bound; µg/kg)	0.0	—	—	—	—	—	—	—	—	—	—	—	0.0	—
	Mean (upper bound; µg/kg)	3.3	—	—	—	—	—	—	—	—	—	—	—	3.3	—
Figs, dried	No. of individual samples	230	—	230	—	—	—	—	—	—	—	—	—	—	—
	% sample < LOD or LOQ	25.2	—	25.2	—	—	—	—	—	—	—	—	—	—	—
	Mean (lower bound; µg/kg)	238.5	—	238.5	—	—	—	—	—	—	—	—	—	—	—
	Mean (upper bound; µg/kg)	250.1	—	250.1	—	—	—	—	—	—	—	—	—	—	—
Groundnuts, shelled	No. of individual samples	16	16	—	—	—	—	—	—	—	—	—	—	—	—
	% sample < LOD or LOQ	81.3	81.3	—	—	—	—	—	—	—	—	—	—	—	—
	Mean (lower bound; µg/kg)	97.0	97.0	—	—	—	—	—	—	—	—	—	—	—	—
	Mean (upper bound; µg/kg)	105.1	105.1	—	—	—	—	—	—	—	—	—	—	—	—
Maize	No. of individual samples	8569	280	519	50	172	289	—	782	808	613	337	2583	1224	912
	% sample < LOD or LOQ	29.8	5.0	64.1	56.0	22.1	41.5	—	31.4	60.9	50.3	23.0	2.8	61.7	7.5
	Mean (lower bound; µg/kg)	1236.9	4322.1	84.2	578.4	2241.1	277.9	—	2971.4	495.1	264.2	634.5	1675.0	98.2	1430.4
	Mean (upper bound; µg/kg)	1260.2	4322.6	92.5	595.2	2243.4	300.0	—	2981.6	677.8	284.3	645.6	1675.6	106.1	1435.7

Group	Statistic[a]	Global total[b]	GEMS/Food clusters												
			A	B	C	D	E	F	G	H	I	J	K	L	M
Millet	No. of individual samples	42	—	—	—	—	—	—	—	—	—	—	—	42	—
	% sample < LOD or LOQ	85.7	—	—	—	—	—	—	—	—	—	—	—	85.7	—
	Mean (lower bound; µg/kg)	0.1	—	—	—	—	—	—	—	—	—	—	—	0.1	—
	Mean (upper bound; µg/kg)	8.4	—	—	—	—	—	—	—	—	—	—	—	8.4	—
Oats	No. of individual samples	17	—	—	—	—	—	—	—	—	—	—	—	—	17
	% sample < LOD or LOQ	88.2	—	—	—	—	—	—	—	—	—	—	—	—	88.2
	Mean (lower bound; µg/kg)	1.5	—	—	—	—	—	—	—	—	—	—	—	—	1.5
	Mean (upper bound; µg/kg)	10.3	—	—	—	—	—	—	—	—	—	—	—	—	10.3
Rice	No. of individual samples	242	—	—	—	—	—	—	—	—	—	21	—	191	30
	% sample < LOD or LOQ	96.3	—	—	—	—	—	—	—	—	—	85.7	—	98.9	86.7
	Mean (lower bound; µg/kg)	1.6	—	—	—	—	—	—	—	—	—	0.1	—	0.0	13.1
	Mean (upper bound; µg/kg)	30.9	—	—	—	—	—	—	—	—	—	8.7	—	22.6	99.8
Sorghum	No. of individual samples	17	—	—	—	—	—	—	—	—	13	—	—	—	4
	% sample < LOD or LOQ	76.5	—	—	—	—	—	—	—	—	100.0	—	—	—	0.0
	Mean (lower bound; µg/kg)	35.5	—	—	—	—	—	—	—	—	0.0	—	—	—	150.8
	Mean (upper bound; µg/kg)	73.7	—	—	—	—	—	—	—	—	50.0	—	—	—	150.8
Soya bean (dry)	No. of individual samples	137	5	—	—	—	—	—	—	—	—	—	—	82	50
	% sample < LOD or LOQ	52.5	60.0	—	—	—	—	—	—	—	—	—	—	84.1	0.0
	Mean (lower bound; µg/kg)	33.1	78.0	—	—	—	—	—	—	—	—	—	—	0.1	82.8
	Mean (upper bound; µg/kg)	34.4	84.0	—	—	—	—	—	—	—	—	—	—	1.8	82.8

Table 13 (contd)

Group	Statistic[a]	Global total[b]	GEMS/Food clusters												
			A	B	C	D	E	F	G	H	I	J	K	L	M
Sweet corn, kernels	No. of individual samples	740	—	71	—	—	—	—	5	—	—	—	122	538	4
	% sample < LOD or LOQ	65.7	—	43.7	—	—	—	—	100.0	—	—	—	38.7	74.2	100.0
	Mean (lower bound; µg/kg)	83.5	—	84.1	—	—	—	—	0.0	—	—	—	389.2	15.5	0.0
	Mean (upper bound; µg/kg)	94.0	—	93.3	—	—	—	—	30.0	—	—	—	397.1	26.2	50.0
Wheat	No. of individual samples	74	—	—	—	—	11	—	—	—	—	—	—	59	4
	% sample < LOD or LOQ	79.7	—	—	—	—	0.0	—	—	—	—	—	—	100.0	0.0
	Mean (lower bound; µg/kg)	37.8	—	—	—	—	220.7	—	—	—	—	—	—	0.0	92.0
	Mean (upper bound; µg/kg)	48.2	—	—	—	—	220.7	—	—	—	—	—	—	13.1	92.0

[a] Two scenarios were considered when calculating the mean values: samples in which the concentration was below the LOQ or LOD were assumed to have a value of either zero (lower-bound scenario) or the limit itself (upper-bound scenario).

[b] The global total lower- and upper-bound averages were obtained by pooling the concentration data across all clusters.

Table 14. Summary of data on concentrations of total fumonisins[a] in commodities from the GEMS/Food clusters

Group	Statistic[b]	Global total	GEMS/Food clusters												
			A	B	C	D	E	F	G	H	I	J	K	L	M
Barley	No. of individual samples	123	—	—	—	—	4	—	25	—	—	—	—	84	10
	% sample < LOD or LOQ	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mean (lower bound; µg/kg)	51.2	—	—	—	—	0.0	—	252.0	—	—	—	—	0.0	0.0
	Mean (upper bound; µg/kg)	101.6	—	—	—	—	200.0	—	340.0	—	—	—	—	35.7	20.0
Buckwheat	No. of individual samples	96	—	—	—	—	—	—	—	—	—	—	—	95	1
	% sample < LOD or LOQ	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mean (lower bound; µg/kg)	0.1	—	—	—	—	—	—	—	—	—	—	—	0.0	5.0
	Mean (upper bound; µg/kg)	9.7	—	—	—	—	—	—	—	—	—	—	—	9.8	5.0
Maize	No. of individual samples	10 759	280	1034	50	172	401		782	808	673	337	3067	1315	1840
	% sample < LOD or LOQ	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mean (lower bound; µg/kg)	1650.8	5920.7	632.1	792.4	3048.6	372.6	—	4070.5	1225.7	420.8	879.1	2472.4	173.5	1180.8
	Mean (upper bound; µg/kg)	1680.6	5921.4	642.4	815.4	3050.6	406.3	—	4088.9	1430.1	451.1	898.3	2474.7	195.7	1208.8
Millet	No. of individual samples	42	—	—	—	—	—	—	—	—	—	—	—	42	—
	% sample < LOD or LOQ	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mean (lower bound; µg/kg)	0.1	—	—	—	—	—	—	—	—	—	—	—	0.1	—
	Mean (upper bound; µg/kg)	18.4	—	—	—	—	—	—	—	—	—	—	—	18.4	—
Oats	No. of individual samples	26	—	—	—	—	2	—	—	—	—	—	—	—	24
	% sample < LOD or LOQ	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mean (lower bound; µg/kg)	1.2	—	—	—	—	0.0	—	—	—	—	—	—	—	1.3
	Mean (upper bound; µg/kg)	22.5	—	—	—	—	200.0	—	—	—	—	—	—	—	7.8

Table 14 (contd)

Group	Statistic[b]	Global total	GEMS/Food clusters												
			A	B	C	D	E	F	G	H	I	J	K	L	M
Rice	No. of individual samples	207	—	—	—	—	—	—	—	—	—	21	—	103	83
	% sample < LOD or LOQ	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mean (lower bound; µg/kg)	1.4	—	—	—	—	—	—	—	—	—	0.7	—	0.0	3.4
	Mean (upper bound; µg/kg)	59.2	—	—	—	—	—	—	—	—	—	43.3	—	26.0	104.5
Soya bean (dry)	No. of individual samples	106	—	—	—	—	—	—	—	—	—	—	—	82	24
	% sample < LOD or LOQ	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mean (lower bound; µg/kg)	5.7	—	—	—	—	—	—	—	—	—	—	—	0.1	24.8
	Mean (upper bound; µg/kg)	11.2	—	—	—	—	—	—	—	—	—	—	—	5.7	29.8
Sweet corn, kernels	No. of individual samples	939	—	71	—	—	12	—	5	—	—	—	122	578	151
	% sample < LOD or LOQ	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mean (lower bound; µg/kg)	131.0	—	92.2	—	—	117.4	—	0.0	—	—	—	538.5	19.6	251.8
	Mean (upper bound; µg/kg)	163.5	—	116.0	—	—	125.7	—	100.0	—	—	—	549.2	51.0	310.0
Wheat	No. of individual samples	94	—	—	—	—	6	—	—	—	—	—	—	59	29
	% sample < LOD or LOQ	—	—	—	—	—	—	—	—	—	—	—	—	—	—
	Mean (lower bound; µg/kg)	0.2	—	—	—	—	0.0	—	—	—	—	—	—	0.0	0.5
	Mean (upper bound; µg/kg)	29.3	—	—	—	—	200.0	—	—	—	—	—	—	24.5	3.8

[a] When not reported, the concentration of total fumonisins was estimated from the concentration of FB_1, FB_2 and, where available, FB_3.

[b] Two scenarios were considered when calculating the mean values: samples in which the concentration was below the LOQ or LOD were assumed to have a value of either zero (lower-bound scenario) or the limit itself (upper-bound scenario).

"soya bean (dry)" and oats from 2 and in millet from only 1. No information on total fumonisins in "figs, dried", sorghum or "groundnuts, shelled" was available. The large majority of the samples considered in the assessment of exposure (87%) referred to maize, followed by "sweet corn, kernels" (8%). Also in the case of total fumonisins, levels in maize vary widely between and within the GEMS/Food clusters (Table 14). Average total fumonisin concentrations in maize ranged, across the different clusters, from 174 to 5921 µg/kg and from 196 to 5921 µg/kg under the lower- and upper-bound scenarios, respectively. Relatively lower levels were detected in "sweet corn, kernels" in cluster K (539–549 µg/kg, lower- and upper-bound means) and cluster M (252–310 µg/kg) and in barley in cluster G (252–340 µg/kg). Upper-bound mean levels of total fumonisins in all other commodities and clusters did not exceed 200 µg/kg.

8.2 International estimates of dietary exposure

The Committee calculated international estimates of dietary exposure to FB_1 and total fumonisins using the GEMS/Food consumption cluster diets (Annex 1, reference *176*). The consumption cluster diets provide mean per capita consumption values based on FAO food balance sheet data for raw commodities and some semi-processed commodities for 13 clusters of countries. Clusters A, I and J include primarily African countries; clusters B and D include countries in West Asia and Europe; cluster C includes countries in Asia and Africa; clusters E and F include European countries; cluster G includes Asian countries; cluster H consists mainly of South American countries; cluster K includes both North and South American countries; cluster L includes Asia/Pacific countries; and cluster M includes Australia/New Zealand/USA/Canada and some South American countries. The countries belonging to each cluster may be found at http://www.who.int/foodsafety/chem/countries.pdf.

Individual data points on the concentration of the contaminant in foods from each cluster are usually pooled to derive summary representative concentrations for each cluster for use in the dietary exposure calculations. For each commodity, when concentration data were not available for a cluster, the global total lower- and upper-bound averages, obtained by pooling the data across all clusters, were used to assess exposure. By doing this, it is assumed that it is a global market and that concentrations from commodities grown in one area of the world are representative of other areas of the world. No weightings were applied to the concentrations, as insufficient information about many of the data was available to allow this to be done. A standard body weight of 60 kg was used to assess exposure per kilogram of body weight.

Consumption data and exposure estimates for FB_1 and total fumonisins are shown in Tables 15 and 16, respectively. Exposures estimated are mean exposures in micrograms per kilogram body weight per day and are representative of chronic dietary exposures.

When the upper-bound scenario was considered, average chronic exposure to FB_1 and total fumonisins, based on the 13 clusters, ranged from 0.3 µg/kg bw per day (cluster L) to 6.2 µg/kg bw per day (cluster A) and from 0.4 µg/kg bw per day (clusters F and L) to 8.4 µg/kg bw per day (cluster A), respectively. No significant

Table 15. Chronic dietary exposure to fumonisin B_1 according to commodities from the GEMS/Food clusters

Group	Statistic[a]	GEMS/Food clusters												
		A	B	C	D	E	F	G	H	I	J	K	L	M
All commodities	Lower bound (µg/kg bw per day)	6.1	0.5	1.7	1.5	1.1	0.3	1.9	2.6	1.2	0.7	1.9	0.1	2.7
	Upper bound (µg/kg bw per day)	6.2	0.6	1.8	1.5	1.1	0.4	2.1	3.6	1.3	0.9	2.1	0.3	2.8
Barley	GEMS/Food consumption (g/day)	40.6	16.8	93.9	13.2	48.6	36.1	5.9	20.5	5.9	2.5	20.2	16.8	43.8
	Lower bound (µg/kg bw per day)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
	Upper bound (µg/kg bw per day)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
Buckwheat	GEMS/Food consumption (g/day)	0.0	0.1	0.0	1.7	1.6	0.1	1.0	0.0	0.2	0.1	0.5	2.0	0.1
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Figs, dried	GEMS/Food consumption (g/day)	0.0	0.6	0.4	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.1
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Groundnuts, shelled	GEMS/Food consumption (g/day)	5.4	3.1	2.1	0.7	4.0	1.4	7.6	2.1	4.7	21.8	0.9	0.7	6.9
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maize	GEMS/Food consumption (g/day)	82.7	148.4	135.9	31.8	33.3	7.5	35.2	298.6	248.1	57.4	63.1	58.6	85.5
	Lower bound (µg/kg bw per day)	6.0	0.2	1.3	1.2	0.2	0.2	1.7	2.5	1.1	0.6	1.8	0.1	2.0
	Upper bound (µg/kg bw per day)	6.0	0.2	1.3	1.2	0.2	0.2	1.7	3.4	1.2	0.6	1.8	0.1	2.0
Millet	GEMS/Food consumption (g/day)	15.8	0.1	0.8	5.6	0.2	0.1	13.0	0.0	8.3	96.9	0.0	0.4	0.0
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Group	Statistic[a]	GEMS/Food clusters												
		A	B	C	D	E	F	G	H	I	J	K	L	M
Oats	GEMS/Food consumption (g/day)	1.4	0.6	0.2	4.2	5.7	8.9	0.2	2.0	0.8	0.0	3.5	0.7	7.6
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rice	GEMS/Food consumption (g/day)	91.0	31.6	94.6	33.2	12.7	12.7	376.9	64.3	38.0	74.3	238.4	381.3	34.6
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.1	0.1	0.1
Sorghum	GEMS/Food consumption (g/day)	36.9	0.0	10.2	0.0	0.0	0.0	9.8	19.9	18.6	112.3	0.1	3.3	3.0
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0
Soya bean (dry)	GEMS/Food consumption (g/day)	9.9	36.4	34.3	22.4	35.3	39.2	25.9	59.4	11.2	11.0	109.3	51.5	123.2
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.2
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.2
Sweet corn, kernels	GEMS/Food consumption (g/day)	14.7	2.0	0.2	1.2	6.5	7.2	0.4	4.9	4.5	3.3	1.7	5.6	18.1
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Wheat	GEMS/Food consumption (g/day)	88.4	396.3	426.5	390.2	236.3	216.0	172.9	79.0	68.1	41.9	114.1	103.4	234.2
	Lower bound (µg/kg bw per day)	0.1	0.2	0.3	0.2	0.9	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.4
	Upper bound (µg/kg bw per day)	0.1	0.3	0.3	0.3	0.9	0.2	0.1	0.1	0.1	0.0	0.1	0.0	0.4

[a] Two scenarios were considered when calculating the mean values: samples in which the concentration was below the LOQ or LOD were assumed to have a value of either zero (lower-bound scenario) or the limit itself (upper-bound scenario).

Table 16. Chronic dietary exposure to total fumonisins according to commodities from the GEMS/Food clusters

Group	Statistic[a]	GEMS/Food clusters												
		A	B	C	D	E	F	G	H	I	J	K	L	M
All commodities	Lower bound (µg/kg bw per day)	8.2	1.6	1.9	1.6	0.2	0.3	2.4	6.1	1.8	0.9	2.6	0.2	1.8
	Upper bound (µg/kg bw per day)	8.4	1.9	2.3	1.9	1.2	0.4	2.9	7.3	2.0	1.0	3.0	0.4	2.0
Barley	GEMS/Food consumption (g/day)	40.6	16.8	93.9	13.2	48.6	36.1	5.9	20.5	5.9	2.5	20.2	16.8	43.8
	Lower bound (µg/kg bw per day)	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.1	0.0	0.2	0.0	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Buckwheat	GEMS/Food consumption (g/day)	0.0	0.1	0.0	1.7	1.6	0.1	1.0	0.0	0.2	0.1	0.5	2.0	0.1
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Maize	GEMS/Food consumption (g/day)	82.7	148.4	135.9	31.8	33.3	7.5	35.2	298.6	248.1	57.4	63.1	58.6	85.5
	Lower bound (µg/kg bw per day)	8.2	1.6	1.8	1.6	0.2	0.2	2.4	6.1	1.7	0.8	2.6	0.2	1.7
	Upper bound (µg/kg bw per day)	8.2	1.6	1.8	1.6	0.2	0.2	2.4	7.1	1.9	0.9	2.6	0.2	1.7
Millet	GEMS/Food consumption (g/day)	15.8	0.1	0.8	5.6	0.2	0.1	13.0	0.0	8.3	96.9	0.0	0.4	0.0
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oats	GEMS/Food consumption (g/day)	1.4	0.6	0.2	4.2	5.7	8.9	0.2	2.0	0.8	0.0	3.5	0.7	7.6
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Group	Statistic[a]	GEMS/Food clusters												
		A	B	C	D	E	F	G	H	I	J	K	L	M
Rice	GEMS/Diet consumption (g/day)	91.0	31.6	94.6	33.2	12.7	12.7	376.9	64.3	38.0	74.3	238.4	381.3	34.6
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.1	0.0	0.1	0.0	0.0	0.0	0.4	0.1	0.0	0.1	0.2	0.2	0.1
Soya bean (dry)	GEMS/Food consumption (g/day)	9.9	36.4	34.3	22.4	35.3	39.2	25.9	59.4	11.2	11.0	109.3	51.5	123.2
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Sweet corn, kernels	GEMS/Food consumption (g/day)	14.7	2.0	0.2	1.2	6.5	7.2	0.4	4.9	4.5	3.3	1.7	5.6	18.1
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
	Upper bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1
Wheat	GEMS/Food consumption (g/day)	88.4	396.3	426.5	390.2	236.3	216.0	172.9	79.0	68.1	41.9	114.1	103.4	234.2
	Lower bound (µg/kg bw per day)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Upper bound (µg/kg bw per day)	0.0	0.2	0.2	0.2	0.8	0.1	0.1	0.0	0.0	0.0	0.1	0.0	0.0

[a] Two scenarios were considered when calculating the mean values: samples in which the concentration was below the LOQ or LOD were assumed to have a value of either zero (lower-bound scenario) or the limit itself (upper-bound scenario).

differences appeared under the lower-bound scenario. When the upper-bound scenario was considered, the maize contribution to the total exposure ranged from 15% to 96% for FB_1 and from 18% to 98% for total fumonisins. Other major contributors were "sweet corn, kernels" and, in clusters with the lowest exposures, wheat.

8.3 National estimates of dietary exposure

Since the evaluation of fumonisins at the fifty-sixth meeting of the Committee in 2001, a number of national evaluations of dietary exposure have been published. The Committee considered evaluations by Brazil, China, the European Union (collectively), France, Guatemala, Italy, the Netherlands, Portugal, the Republic of Korea, South Africa, Spain, the United Republic of Tanzania and the USA (Table 17). Most of these reports contained overall dietary exposure assessments for FB_1 only (six studies); exposure to FB_2 and FB_3 was assessed only in China. "Total fumonisins" was expressed as $FB_1 + FB_2$ and as $FB_1 + FB_2 + FB_3$ in six and seven countries, respectively. Maize was often the only source of fumonisins considered; in some of the studies, other cereals and cereal-based products were also taken into account.

8.3.1 Australia

Fumonisins were analysed as part of the 23rd Australian Total Diet Study. Results of this survey will be published on the Food Standards Australia New Zealand web site (http://www.foodstandards.gov.au) in 2011. However, Food Standards Australia New Zealand indicated to the Committee that, as there were no detections of mycotoxins, including fumonisins (FB_1 and FB_2), in any of the foods analysed, dietary modelling for fumonisins was not undertaken.

8.3.2 Brazil

In Brazil, daily exposure to fumonisins through the consumption of corn-based food products was estimated using consumption data estimated from the 2002/2003 Brazilian Household Budget Survey and the level of fumonisins (FB_1 and FB_2) found in this and other studies conducted in Brazil (Caldas & Silva, 2007). FB_1 and FB_2 were analysed in 208 samples of corn-based products, including two types of cornmeals ("fubá" and "creme de milho"), popcorn, two types of precooked corn flour ("beiju" and "milharina"), corn flakes, corn snacks and sweet corn. Cornmeal samples contained the highest levels of fumonisins; the mean concentration of $FB_1 + FB_2$ was found to be 1680 and 2040 µg/kg for "fubá" and "creme de milho", respectively. The total exposure to fumonisins ($FB_1 + FB_2$) from the consumption of corn-based products in Brazil was estimated to be equal to 0.5 and 7.1 µg/kg bw per day in the total population and consumers only, respectively. Cornmeal contributed the most to the exposure (54–74%), followed by precooked corn flours (18–24%).

8.3.3 China

Two hundred and nine food samples (108 for maize, 29 for rice, 16 for wheat flour, 39 for plant oil and 17 for peanut) were measured for FB_1 within a study aimed at

Table 17. National estimates of dietary exposure to fumonisins

Country/region	Population groups	Food products/main contributors	Total dietary exposure (μg/kg bw per day)		Reference
			Mean/median	High percentile	
FB_1					
China	Regional population	Maize, rice, wheat flour, plant oil and peanut	7.6[a]	33.3	Sun et al. (2011)
China	General population	Total diet study	0.13	—	Submitted to JECFA
EU member states (7)	Infants, children, adolescents and adults	Corn, corn fractions, corn-derived products, cereal products, wheat products, millet, rice and others	0.12×10^{-3}–0.52	—	SCOOP (2003)
Islamic Republic of Iran	Regional population	Maize	9×10^{-3}–0.712	—	Yazdanpanah et al. (2006)
Netherlands	General population	Maize, corn flakes, popcorn, maize meal, white wheat (flour), wheat bran and rice	0.03–0.15[a]	0.10–0.38	Bakker et al. (2003)
Netherlands	Children aged 2–6 years	Maize, sweet corn, children's food (i.e. porridge and children's food in jars) and pasta	0.3[a]	1.0	Boon et al. (2009)
Portugal	General population	Maize	0.051	—	Silva et al. (2007)
USA	Regional pregnant women (case–control study)	Tortilla	0.156[a]–0.173[a]	—	Missmer et al. (2006)
FB_2					
China	General population	Total diet study	0.02	—	Submitted to JECFA
FB_3					
China	General population	Total diet study	0.01	—	Submitted to JECFA

Table 17 (contd)

Country/region	Population groups	Food products/main contributors	Total dietary exposure (µg/kg bw per day)		Reference
			Mean/median	High percentile	
$FB_1 + FB_2$					
Brazil	General population	Corn-based products	0.5–7.1	—	Caldas & Silva (2007)
EU member states (7)	Children, adolescents and adults	Corn, corn fractions, corn-derived products, cereal products, wheat products, millet, rice and others	0.5×10^{-3}–0.35	—	SCOOP (2003)
Portugal	General population	Maize	0.065	—	Silva et al. (2007)
Republic of Korea	Adults	White rice, brown rice, barley, barley tea, beer, millet, wheat flour, dried corn, popcorn, corn flour, corn tea, canned corn, breakfast cereal	0.087×10^{-3}	—	Seo et al. (2009)
South Africa	Regional children, adults and elderly	Maize	3.03–14.14	5.96–27.9	Shephard et al. (2007)
Spain	General population	Organic and conventional corn	3.4×10^{-3}–4.1×10^{-3}	—	Ariño et al. (2007)
United Republic of Tanzania	Regional infants 6–8 months of age	Sorted and unsorted maize	0.47[a]	10.77–37	Kimanya et al. (2009)
Total fumonisins					
China	General population	Total diet study	0.16	—	Submitted to JECFA
France	Children, adults and vegetarians	Total diet study	0.014–0.046	0.064–0.29[b]	Leblanc et al. (2005)

Country/region	Population groups	Food products/main contributors	Total dietary exposure (µg/kg bw per day)		Reference
			Mean/median	High percentile	
Guatemala[c]	Regional adults, women	Nixtamalized maize products	2.2–10.6	10.6–44.8	Torres et al. (2007)
Italy	Infants and adults	Baby food, corn flour, corn flakes, pasta, cookies and other corn products (organic and conventional)	5×10^{-3}–20×10^{-3}	—	D'Arco et al. (2009)
South Africa	Adults	Home-grown and commercial maize and home-brewed maize-based beer	1.1–8.5	12.0[d]	Burger et al. (2010)
Spain	Infants and adults	Baby food, corn flour, corn flakes, pasta, cookies and other corn products (organic and conventional)	1.7×10^{-3}–0.72	—	D'Arco et al. (2009)
United Republic of Tanzania	Infants 6–8 months of age	Maize	0.48[a]	3.99	Kimanya et al. (2010)

EU, European Union

[a] Median (all others are means).

[b] 95th percentile among vegetarians.

[c] The original total fumonisin exposure included FB_1, FB_2, FB_3, HFB_1, HFB_2 and HFB_3. The hydrolysed fumonisins accounted for approximately 50% of the total fumonisin exposure on a molar basis.

[d] Traditional home-brewed maize-based beer consumers only.

investigating co-contamination of AFB_1 and FB_1 in food and human dietary exposure to these mycotoxins in residents of three different areas of China (Sun et al., 2011). High contamination rates of FB_1 were found in cereal grain samples, including corn, rice and wheat flour, from these three areas. About 58% of Huaian corn samples had an FB_1 level that exceeded 2000 µg/kg. Based on food consumption records and FB_1 concentrations in foods, the daily dietary exposure to FB_1 was estimated for the residents of these three areas. FB_1 contamination in foods and the daily dietary exposure in residents of Huaian were the highest among these three areas; median and 95th percentile exposures were estimated to be equal to 7.6 and 33.3 µg/kg bw per day, respectively (assuming a standard body weight of 60 kg).

Dietary exposure of the Chinese population to fumonisins was estimated within the 2007 Chinese Total Diet Study (data submitted to JECFA). In this study, 665 composite samples were analysed as cooked. The estimated average total exposure to fumonisins was 0.13, 0.02 and 0.01 µg/kg bw per day for FB_1, FB_2 and FB_3, respectively. Cereals were the major contributor to fumonisin exposure in the country.

8.3.4 European Union

In 2003, the European Commission completed a Scientific Cooperation (SCOOP) task, which involved collection of occurrence data on fumonisins in food and assessment of dietary exposures for the population of the European Union member states (SCOOP, 2003). Occurrence data have been provided by nine countries for FB_1 (Austria, Belgium, France, Germany, Italy, the Netherlands, Norway, Sweden and the United Kingdom), six countries for FB_2 (Austria, France, Italy, Norway, Sweden and the United Kingdom) and one for FB_3 (the United Kingdom). Results for a total of 5112 samples (3863 for FB_1, 1010 for FB_2 and 239 for FB_3) were reported. Germany (51%), France (20%) and Italy (10%) submitted the majority of the analytical data for FB_1, whereas data for FB_2 were mainly received from France (35%), the United Kingdom (24%) and Norway (20%). Dietary exposure to FB_1 and $FB_1 + FB_2$ was estimated in seven countries (Austria, Belgium, France, Germany, Italy, the Netherlands and Norway) also providing appropriate food consumption data.

The total average dietary exposure was obtained by summing up the average exposure estimates from all commodities under evaluation (corn, corn fractions, corn-derived products, cereal products, wheat products, millet, rice and others). Mean total dietary exposure to FB_1 ranged from 0.12×10^{-3} µg/kg bw per day (in Norway) to 0.52 µg/kg bw per day (among Italian consumers) across all countries and age groups considered. Mean total dietary exposure to $FB_1 + FB_2$ ranged from 0.5×10^{-3} µg/kg bw per day (in Norway) to 0.35 µg/kg bw per day (among Italian consumers) across all countries and age groups considered. Exposure to $FB_1 + FB_2$ was also estimated to be equal to 0.43 µg/kg bw per day among infants consuming baby porridge in Norway. No participating country could estimate exposures from all the commodities known to be susceptible to fumonisin contamination. Corn and corn-derived products were the major contributors to dietary exposure for FB_1 and $FB_1 + FB_2$ in all countries with the exception of France, where exposure to fumonisins was mainly due to wheat products.

8.3.5 France

Dietary exposure of the French population to the principal mycotoxins in the French diet was estimated within the first French Total Diet Study (Leblanc et al., 2005). In this study, 456 composite samples were prepared from 2280 individual samples and analysed for aflatoxins, OTA, trichothecenes, ZEA, fumonisins and patulin. Average and high-percentile intakes were calculated taking account of different eating patterns for adults, children and vegetarians. The results show that 12 of 34 composite samples exhibited levels above the LOD for FB_1, and 1 sample was above the LOD for FB_2. Five out of the 12 samples positive for FB_1 were breakfast cereals (42%) with concentrations between 60 and 120 µg/kg, the remainder being three poultry liver samples (50% of all the poultry liver samples analysed) with a content of FB_1 between 90 and 120 µg/kg. The estimated average total exposure to fumonisins in the French population was equal to 14×10^{-3} µg/kg bw per day in adults and 46×10^{-3} µg/kg bw per day in children. The 95th percentile exposure was 64×10^{-3} µg/kg bw per day in adults and 0.175 µg/kg bw per day in children. When vegetarians were considered, the 95th percentile exposure was estimated to be equal to 0.29 µg/kg bw per day.

8.3.6 Guatemala

Analysis of maize samples (n = 396) collected from fields in Guatemala from 2000 to 2003 found that lowland maize (<360 m) had significantly more FB_1 than highland maize (>1200 m) (Torres et al., 2007). Based on a recall study and published consumption data, a preliminary assessment of daily exposure to total fumonisins from maize was made. The original total fumonisin exposure included FB_1, FB_2, FB_3, HFB_1, HFB_2 and HFB_3. The hydrolysed fumonisins, accounting for approximately 50% of the total fumonisin exposure on a molar basis, were removed in order to allow comparison with other national exposure assessments. Average fumonisin ($FB_1 + FB_2 + FB_3$) exposure was calculated to range from 2.2 to 10.6 µg/kg bw per day across the different regions and refers to women eating nixtamalized maize products (from 102 to 454 g/day) made from maize sold in local markets containing fumonisins, including hydrolysed fumonisins, at a level of 3550 µg/kg. The highest level of fumonisin exposure was estimated to be equal to 44.8 µg/kg bw per day and is based on a level of fumonisins, including the hydrolysed form, of 18 280 µg/kg.

8.3.7 Islamic Republic of Iran

A study was undertaken in the Islamic Republic of Iran to investigate the variation in levels of contamination and to estimate possible levels of human exposure to fumonisins in the country (Yazdanpanah et al., 2006). The mean level of FB_1 in 49 maize samples collected from Mazandaran Province during 2000 was 6140 µg/kg, which is higher than those concentrations found during 1998 and 1999 (2270 and 3180 µg/kg, respectively). Exposure to FB_1 was estimated using maize consumption data from the FAO food balance sheets (3.3 g per person per day in 2002) and assuming an adult body weight of 60 kg. Per capita exposure to FB_1 ranged, across the different regions considered, from 9×10^{-3} to 0.338 µg/kg bw per day, assuming an average concentration, and from 32×10^{-3} to 0.712 µg/kg bw per day, when the maximum FB_1 value was used.

8.3.8 Italy and Spain

In 2007, an ad hoc survey was conducted in Italy and Spain to determine the occurrence of FB_1, FB_2 and FB_3 in 186 samples of organic and conventional locally available corn products (D'Arco et al., 2009). Samples including baby food ($n = 62$), corn flour ($n = 11$), corn flakes ($n = 23$), pasta ($n = 14$), cookies ($n = 17$) and other corn products ($n = 59$) were obtained from popular markets of Valencia (Spain) and Perugia (Italy). Exposure to fumonisins through corn was estimated in infants and adults considering organic or conventional products separately. Exposure ranged from 1.7×10^{-3} to 0.72 µg/kg bw per day in Spain and from 5×10^{-3} to 20×10^{-3} µg/kg bw per day in Italy.

8.3.9 Netherlands

The intake of FB_1 by the population in the Netherlands was estimated in an exposure assessment (Bakker et al., 2003) that used Dutch FB_1 occurrence data, according to seven food groups (maize, corn flakes, popcorn, maize meal, white wheat [flour], wheat bran and rice), combined with those sampled in other European Union countries, as reported by the above-mentioned SCOOP (2003) report. Data from the Dutch National Food Consumption Survey, including information on the daily consumption over 2 consecutive days and a record of age, sex and body weight for 6250 individuals, were used for this assessment. Average FB_1 exposure was estimated to be 0.05 µg/kg bw per day. When correcting for the between-days variation in food consumption, the lifetime-averaged median and 99th percentile exposures were equal to 0.03 and 0.10 µg/kg bw per day, respectively. A worst-case calculation resulted in lifetime-averaged median and 99th percentile exposures of 0.15 and 0.38 µg/kg bw per day. The majority of the exposure to FB_1 occurred via the consumption of wheat, although the concentration of FB_1 in maize was much higher.

Dietary exposure to fumonisins among children in the Netherlands was estimated in another report (Boon et al., 2009). For this purpose, food consumption data from the Dutch National Food Consumption Survey 2005/2006 (Young children) were linked to data on the concentration of FB_1 in foods. Median and 99th percentile FB_1 exposures of children aged 2–6 years were estimated to be 0.3 and 1.0 µg/kg bw per day, respectively. The main food groups contributing to the exposure were maize, sweet corn, children's food (i.e. porridge and children's food in jars) and pasta.

In the National Institute for Public Health and the Environment's children's duplicate diet study of 2006, FB_1 was detected in 28% of the 123 samples (Van Egmond, 2008). The resulting estimated exposures from the positive samples varied between 8×10^{-3} and 0.288 µg/kg bw per day.

8.3.10 Portugal

A survey was conducted on the incidences of FB_1 and FB_2 in both maize and maize-derived products purchased in Portugal (Silva et al., 2007). The presence of FB_1 and FB_2 was determined in 67 samples of maize and maize-based foods, such as flour, semolina, starch, sweet maize, corn flakes and other breakfast cereals, and snacks collected in 2005. The average sample contamination of FB_1 obtained in the present study was 110 µg/kg, and for $FB_1 + FB_2$, it was 140 µg/kg. Exposure to fumonisins was estimated by using maize consumption data from the Portuguese

food balance sheets (27.9 g per person per day in 2003) and assuming an adult body weight of 60 kg. Per capita exposure to FB_1 and $FB_1 + FB_2$ in the Portuguese population was then estimated to be equal to 51×10^{-3} and 65×10^{-3} µg/kg bw per day, respectively.

8.3.11 Republic of Korea

Daily exposure to fumonisins was estimated in the Republic of Korea by combining analytical data on FB_1 and FB_2 in white rice, brown rice, barley, barley tea, beer, millet, wheat flour, dried corn, popcorn, corn flour, corn tea, canned corn and breakfast cereal (12 samples for each food commodity, for a total of 156 samples) with average, nationally representative food consumption and body weight values (Seo et al., 2009). Only FB_1-positive samples were found, three dried corn samples and five corn flour samples. The estimated average daily intake of $FB_1 + FB_2$ was 0.087×10^{-3} µg/kg bw. Dried corn was the only source of exposure in the Republic of Korea, as information on the consumption of corn flour was not available.

8.3.12 South Africa

Shephard et al. (2007) assessed exposure to fumonisins in two regions of South Africa (Bizana and Centane) by combining maize consumption data, collected at the household level within an ad hoc study including a total of 922 subjects, with previously determined levels of total fumonisin (FB_1 and FB_2 combined) contamination in home-grown maize. Maize consumption was reported according to region, sex and age class (from 1–9 years old to >65 years old) and ranged from 231 to 530 g/day and from 391 to 783 g/day for the average and 95th percentile, respectively. Fumonisin exposure ranged from 3.03 to 14.14 µg/kg bw per day and from 5.96 to 27.9 µg/kg bw per day for the average and 95th percentile, respectively.

The habitual maize intakes were also assessed in 517 subjects living in the Centane region (Burger et al., 2010). Only 2% ($n = 5$) of the population consumed home-grown maize as the main source of their daily diet, and the total mean maize intakes ranged between 400 and 600 g/day. As in the study from Shephard et al. (2007), dietary exposure was assessed by assuming mean total fumonisins ($FB_1 + FB_2 + FB_3$) in maize at 1142 and 222 µg/kg for home-grown and commercial maize, respectively. Subjects consuming home-grown and commercial maize were exposed to fumonisins, on average, at a level of 8.5 and 1.1 µg/kg bw per day, respectively. The contribution of traditional home-brewed maize-based beer was also assessed. The highest probable exposure to fumonisins from this product was estimated to be equal to 12.0 µg/kg bw per single drinking event.

8.3.13 Spain

Dietary exposure to fumonisins was assessed by Ariño et al. (2007) based on analytical data from 60 samples of conventional and organic corn. FB_1 was detected above the LOQ in four samples of conventional corn (13.3%) and in three samples of organic corn (10.0%), whereas FB_2 occurred in 10% of 30 samples of conventional corn and in 6.7% of 30 samples of organic corn. The average levels of FB_1 and FB_2 in conventional corn samples amounted to 43.2×10^{-3} and 21.9×10^{-3} µg/kg, respectively, whereas organic corn samples showed somewhat lower levels

of contamination: 35.4×10^{-3} and 18.8×10^{-3} µg/kg for FB_1 and FB_2, respectively. An estimation of dietary exposure to fumonisins from conventional and organic corn was carried out by using corn consumption data from the latest food supply and commodity balance data (4.4 g per person per day) and assuming an adult body weight of 70 kg. Per capita exposure to $FB_1 + FB_2$ in the Spanish population from conventional and organic corn was then estimated to be equal to 4.1×10^{-3} and 3.4×10^{-3} µg/kg bw per day, respectively.

8.3.14 United Republic of Tanzania

Exposure to fumonisins ($FB_1 + FB_2$) from maize was assessed in infants 6–8 months of age in the United Republic of Tanzania (Kimanya et al., 2009). Maize consumption, estimated by twice conducting a 24-hour dietary recall for 254 infants, ranged from 2.37 to 158 g/day (43 g/day on average) among the 226 consumers (89% of the population); 4% of the infants consumed maize at levels above 100 g of maize per person per day. Fumonisin exposure was modelled by multiplying maize meal consumption data with different contamination distributions, as determined in samples of sorted and unsorted maize from 2005 and 2006. When the pooled contamination data sets for maize in 2005 and 2006 were considered, median and 97th percentile exposures were equal to 0.47 and 10.77 µg/kg bw per day, respectively. This is the most representative scenario for infants in this community according to the authors, but the 97th percentile of exposure was also estimated at a level of 37 µg/kg bw per day based on contamination data from sorted maize from 2005. The authors highlighted the fact that records of complementary food consumption in the United Republic of Tanzania indicate that from 9 to 10 months of age, the variety of foods fed to breastfeeding children expands. Although foods made from grains are still the most common, some children start receiving other foods, including fruits and vegetables, other milk products, meat, fish and poultry.

Dietary exposure to fumonisins ($FB_1 + FB_2 + FB_3$) was also estimated within a study in the United Republic of Tanzania aimed at evaluating a dose–effect relationship between fumonisin exposure and growth retardation in infants (Kimanya et al., 2010). Maize consumption, estimated by twice conducting a 24-hour dietary recall for 215 infants, ranged from 1 to 106 g/day (32 g/day on average) among the 191 consumers (89% of the population). Fumonisin exposure for each child was estimated by combining his/her maize intake and the fumonisin level in the maize. Of the 191 samples of maize flour, 131 (69%) contained total fumonisins ($FB_1 + FB_2 + FB_3$) at levels varying from 21 to 3201 µg/kg (median 158 µg/kg). Median and 90th percentile fumonisin ($FB_1 + FB_2 + FB_3$) exposures of infants were estimated at 0.48 and 3.99 µg/kg bw per day, respectively. Although not significant, a definite decrease in infant length was observed with increase in fumonisin exposure.

8.3.15 USA

Missmer et al. (2006) assessed exposure to fumonisins in a sample of pregnant Mexican American women who resided and delivered in one of the Texas–Mexico border counties. This study was aimed at examining whether or not maternal exposure to fumonisins increases the risk of NTDs in offspring using a population-based case–control study. Women were specifically asked about corn

tortilla consumption (frequency and quantity) during preconception, including the type (brand name, homemade). To estimate fumonisin levels in tortillas, samples of tortillas from participant homes (146 samples) and local grocery stores (114 samples) were collected and analysed for FB_1. The mean FB_1 level measured in the tortilla samples was 234 µg/kg (range = 0–1690 µg/kg; SD = 256 µg/kg). Nearly all women consumed corn tortillas (96% of cases and 93% of controls). Median FB_1 exposure was 0.173 and 0.156 µg/kg bw per day for cases and controls, respectively. Corn tortilla and flour samples (n = 38) from Los Angeles and San Diego (USA) and Tijuana (Mexico) were also analysed for fumonisin contamination (Dvorak et al., 2008). The median FB_1 level was 84 µg/kg (ranging from 2.8 to 1863 µg/kg).

8.4 Potential effect of limits and their enforcement on chronic dietary exposure

The Codex Committee on Contaminants in Foods (CCCF) (FAO/WHO, 2010a) has proposed the establishment of maximum levels (MLs) for fumonisins (FB_1 + FB_2) in maize and maize-based products. In order to evaluate the potential effect of these MLs on chronic dietary exposure, all occurrence data on total fumonisins (as reported or calculated) were categorized into the groups for which an ML has been proposed: "corn/maize grain, unprocessed" (ML = 5000 µg/kg), "corn/maize flour/meal" (ML = 2000 µg/kg), "popcorn grain" (ML = 2000 µg/kg), "maize-based baby food" (ML = 500 µg/kg) and "maize-based breakfast cereals, snacks and chips" (ML = 1000 µg/kg). An international dietary exposure assessment for total fumonisins was performed based on these MLs. For this, all samples for which the upper-bound mean concentration of total fumonisins exceeded its ML were excluded from the calculation.

The percentages of rejected samples after implementation of the proposed MLs are presented, by food category and cluster, in Table 18. MLs did not result in rejected samples in the majority of commodities and clusters. Overall, only 11% of the samples were excluded.

Rejection of samples was noted for "corn/maize flour/meal" in four clusters (from 4% to 57% rejected samples and from 39% to 89% reduction for the upper-bound mean), "corn/maize grain, unprocessed" in six clusters (from 1% to 88% rejected samples and from 7% to 70% reduction for the upper-bound mean), "maize-based breakfast cereals, snacks and chips" in one cluster (11% rejected samples and 30% reduction for the upper-bound mean) and "popcorn grain" in one cluster (17% rejected samples and 40% reduction for the upper-bound mean).

The Committee also evaluated the impact of a range of hypothetical MLs for the categories "corn/maize grain, unprocessed" (10 000, 7000, 5000, 2500, 1000 and 500 µg/kg) and "corn/maize flour/meal" (4000, 3000, 2000, 1000, 500 and 250 µg/kg) on the rejection of samples (Tables 19 and 20).

The effect of the implementation of the proposed Codex MLs, and of the different combinations of MLs for the two above-mentioned food categories, on chronic dietary exposure to total fumonisins was evaluated by means of the GEMS/Food consumption cluster diets (Table 21). For the upper-bound scenario and MLs proposed by Codex, reduction in exposure from all commodities occurred in nine clusters (from 6% to 68%), and the total dietary exposure to total fumonisins ranged from 0.3 µg/kg bw per day (cluster F) to 6.9 µg/kg bw per day (cluster H).

Table 18. Effect of the implementation of the proposed Codex maximum levels in the proposed food categories on the rejection of samples per GEMS/Food cluster

Commodity	GEMS/Food cluster	Number of samples	% of rejected samples after the implementation of Codex MLs
Corn/maize grain, unprocessed	Cluster A	280	88
Corn/maize flour/meal	Cluster G	331	57
Corn/maize flour/meal	Cluster K	721	53
Corn/maize grain, unprocessed	Cluster D	172	28
Popcorn grain	Cluster K	70	17
Corn/maize flour/meal	Cluster B	554	16
Corn/maize grain, unprocessed	Cluster K	2296	12
Maize-based breakfast cereals, snacks and chips	Cluster K	102	11
Corn/maize flour/meal	Cluster M	781	4
Corn/maize grain, unprocessed	Cluster J	337	2
Corn/maize grain, unprocessed	Cluster H	805	2
Corn/maize grain, unprocessed	Cluster M	1047	1

The other exposure estimates were obtained by using different combinations of MLs for the two above-mentioned food categories, whereas for the other food categories, the MLs proposed by CCCF were used. No or little effect was noticed on the international exposure estimates resulting from the implementation of MLs higher than those proposed by CCCF. None of the evaluated MLs, including the one proposed by CCCF for the category "corn/maize flour/meal", produced a relevant reduction in exposure in all clusters. The exposure estimate in cluster M even increased, as a result of the rejection of samples with relatively low fumonisin levels within the GEMS/Food category "maize". MLs of 5000 and 2500 µg/kg for "corn/maize grain, unprocessed" reduced exposure estimates more than 50% in three and four clusters, respectively. The resulting exposure estimates were above 2 µg/kg bw per day in four, one and zero clusters based on hypothetical MLs for "corn/maize grain, unprocessed" at 2500, 1000 and 500 µg/kg, respectively.

9. DOSE–RESPONSE ANALYSIS

9.1 Identification of key data for risk assessment

9.1.1 Pivotal data from biochemical and toxicological studies

In 2001 (Annex 1, reference *153*), the pivotal studies for estimating the PMTDI for FB_1 were selected from a fairly large number of rodent studies conducted with highly purified and well-characterized FB_1 (Annex 1, reference *153*, Tables 21

Table 19. Effect of the implementation of a range of hypothetical maximum levels for "corn/maize grain, unprocessed" on the rejection of samples per GEMS/Food cluster

GEMS/ Food cluster	Number of samples	% of rejected samples after the implementation of MLs					
		500 µg/kg	1000 µg/kg	2500 µg/kg	5000 µg/kg	7000 µg/kg	10 000 µg/kg
Cluster A	280	100	96	88	88	3	—
Cluster B	300	31	31	31	—	—	—
Cluster C	20	100	100	—	—	—	—
Cluster D	172	51	51	40	28	28	6
Cluster E	96	58	58	—	—	—	—
Cluster F	0	—	—	—	—	—	—
Cluster G	431	60	49	27	—	—	—
Cluster H	805	90	31	31	2	—	—
Cluster I	572	29	22	—	—	—	—
Cluster J	337	27	27	9	2	—	—
Cluster K	2296	100	95	32	12	3	—
Cluster L	339	8	8	—	—	—	—
Cluster M	1047	88	33	33	1	1	1

and 22). NOAELs and LOAELs based on oral (gavage or in defined diet) exposure were available from 11 studies conducted in B6C3F1 mice and four different strains of rats, with several studies conducted using both males and females. Both hepatic and renal effects were considered and modelled for dose–response relationships for liver cancer (female mice; NTP, 2001) and kidney cancer (male rats; NTP, 2001). Ultimately, the PMTDI was estimated based on renal toxicity in male Fischer 344N rats (Voss et al.,1995; NTP, 2001).

There are few new dose–response feeding studies conducted using highly purified FB_1. However, there have been several dose–response feeding studies conducted using *F. verticillioides* culture material amended into defined diets. The selection criteria for the present evaluation were similar to those of the previous evaluation. Studies selected for modelling the effects of FB_1 itself were to be dose–response oral exposure, preferably feeding, studies using defined diets containing either pure or purified (>90%) FB_1 and indicating a clear dose–response relationship based on a quantifiable index (e.g. pathology scores). In addition, recognizing that fumonisins occur naturally in maize kernels as a consequence of fungal fermentation in situ, studies using diets prepared with materials in which the fumonisin content was well defined, and meeting all the other criteria for studies with purified FB_1, were also modelled using FB_1 as an indicator of *F. verticillioides* contamination.

Table 20. Effect of the implementation of a range of hypothetical maximum levels for "corn/maize flour/meal" on the rejection of samples per GEMS/ Food cluster

GEMS/ Food cluster	Number of samples	% of rejected samples after the implementation of MLs					
		250 µg/kg	500 µg/kg	1000 µg/kg	2000 µg/kg	3000 µg/kg	4000 µg/kg
Cluster A	0	—	—	—	—	—	—
Cluster B	554	63	42	16	16	—	—
Cluster C	0	—	—	—	—	—	—
Cluster D	0	—	—	—	—	—	—
Cluster E	70	53	53	—	—	—	—
Cluster F	0	—	—	—	—	—	—
Cluster G	331	85	85	85	57	57	43
Cluster H	3	100	100	—	—	—	—
Cluster I	101	9	9	—	—	—	—
Cluster J	0	—	—	—	—	—	—
Cluster K	721	87	85	76	53	32	11
Cluster L	878	15	13	4	—	—	—
Cluster M	781	90	12	4	4	4	2

Based on the criteria described above, the following studies were identified as providing recent additional data for dose–response analysis: Howard et al. (2002), Riley & Voss (2006), Bondy et al. (2010) and Voss et al. (2011). The long-term feeding study conducted by the NTP (2001) was also included as a study for modelling purposes so as to provide a point of comparison of the studies used in the 2001 evaluation (Annex 1, reference *153*) with the studies that met the selection criteria for this evaluation.

The following is a brief description of the critical end-points for the studies meeting the selection criteria described above:

- Howard et al. (2002) conducted a 28-day study in female mice with doses ranging from 0 to 22.9 mg/kg bw per day. Centrilobular apoptosis, hypertrophy and other microscopic changes indicative of liver toxicity at 11.5 mg/kg bw per day were reported.
- Bondy et al. (2010) conducted a 6-month mouse feeding study with male transgenic p53+/– and corresponding wild-type mice and pure FB_1 (0, 5, 50 or 150 mg/kg diet). These diets result in estimated doses of 0, 0.4, 4 and 12 mg/kg bw per day. Pathological examination indicated an increased incidence of megalocytic hepatocytes at 0.4 mg/kg bw per day and increased apoptosis and other microscopic changes indicative of liver toxicity at 4 mg/kg bw per day.

Table 21. Effect of the implementation of a range of hypothetical maximum levels and the proposed Codex maximum levels on the international estimate of chronic dietary exposure to total fumonisins

ML[a] for corn/ maize grain, unprocessed (µg/kg)	ML[a] for corn/maize flour/meal (µg/kg)	Chronic dietary exposure (µg/kg bw per day) by GEMS/Food cluster												
		A	B	C	D	E	F	G	H	I	J	K	L	M
No limits	No limits	8.4	1.9	2.3	1.9	1.2	0.4	2.9	7.3	2.0	1.0	3.0	0.4	2.0
10 000	4000	8.4	1.9	2.3	1.6	1.2	0.4	1.3	7.3	2.0	1.0	2.9	0.4	1.8
10 000	2000	8.4	1.4	2.3	1.6	1.2	0.4	1.2	7.3	2.0	1.0	2.8	0.4	1.8
7000	3000	8.3	1.9	2.3	0.8	1.2	0.4	1.2	7.3	2.0	1.0	2.6	0.4	1.8
5000	2000	2.7	1.4	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.3	0.4	1.8
2500	1000	2.7	0.8	2.3	0.4	1.2	0.3	0.8	3.1	2.0	0.7	2.0	0.4	0.9
1000	500	1.4	0.5	1.5	0.3	1.1	0.2	0.6	3.0	1.1	0.5	0.8	0.3	0.9
500	250	0.4	0.4	1.5	0.3	1.1	0.2	0.5	2.2	1.0	0.5	0.5	0.3	0.6
5000	4000	2.7	1.9	2.3	0.8	1.2	0.3	1.3	6.9	2.0	0.9	2.4	0.4	1.8
5000	3000	2.7	1.9	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.3	0.4	1.8
5000	1000	2.7	1.4	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.3	0.4	1.8
5000	500	2.7	1.3	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.3	0.3	1.8
5000	250	2.7	1.3	2.3	0.8	1.2	0.3	1.2	6.9	2.0	0.9	2.4	0.3	2.3
7000	2000	8.3	1.4	2.3	0.8	1.2	0.4	1.2	7.3	2.0	1.0	2.6	0.4	1.8
2500	2000	2.7	0.8	2.3	0.4	1.2	0.3	0.9	3.1	2.0	0.7	2.0	0.4	0.9
1000	2000	1.4	0.8	1.5	0.3	1.1	0.3	0.8	3.1	1.1	0.5	1.3	0.4	0.9
500	2000	0.6	0.8	1.5	0.3	1.1	0.2	0.7	2.2	1.0	0.5	1.3	0.4	0.7

[a] Proposed MLs by CCCF: 5000 µg/kg for "corn/maize grain, unprocessed", 2000 µg/kg for "corn/maize flour/meal", 2000 µg/kg for "popcorn grain", 500 µg/kg for "maize-based baby food" and 1000 µg/kg for "maize-based breakfast cereals, snacks and chips".

- Riley & Voss (2006) conducted a study in which the effects of 1- to 10-day oral exposure to *F. verticillioides* culture material in the diet were examined in male rats. Biochemical and pathological observations were recorded at 1, 3, 5 and 10 days of exposure with a control group and two dose levels of 0.9 and 5.7 mg/kg bw per day at each time point.
- Voss et al. (2011) conducted an 8-week rat feeding study. Diets prepared with maize grits fermented with *F. verticillioides*, which were subsequently subjected to various processing steps, were found to contain <0.1, 0.3, 1.4, 2.9, 4.9, 9 and 25 mg FB_1 per kilogram diet. Treatment-related tissue lesions (increased apoptosis, tubular necrosis, mitotic figures) were limited to the kidney. Observations were recorded after both 3 and 8 weeks of exposure.

In addition to the new studies identified above, renal toxicity and tumour incidence data from the long-term NTP feeding study (NTP, 2001) were used for modelling and also included as a pivotal study. This study also meets the selection criteria.

9.1.2 Pivotal data from human clinical/epidemiological studies

No pivotal data were selected from the epidemiological studies for dose–response analysis.

9.2 General modelling considerations

9.2.1 Dose–response modelling and BMD calculations

Dose–response modelling was carried out using the United States Environmental Protection Agency's (USEPA) benchmark dose (BMD) software (BMDS version 2.1.2). The nine different dichotomous models were fit to the data selected for modelling. When applicable, exponential shape parameters were constrained to be above 1 in order to avoid supalinear model forms. With the exception of the log-probit model, these are constrained by default. Potential background responses were modelled as extra risk.

The reported *P*-values were used to evaluate model goodness of fit. The Committee considered that a relative difference in *P*-values of 3 would be a suitable selection criterion for the assessment of fumonisin at the present meeting. Application of a relative factor of 3 results in the exclusion of lower limit on the benchmark dose (BMDL) values derived by models with *P*-values that are 3-fold, or more, lower than the highest *P*-value.

9.2.2 Selection of data

Because differences in interpretation are necessary, the data from studies identified as having data suitable for BMD estimation that were conducted with purified FB_1 are reported separately from those conducted with either culture material or naturally contaminated corn. As many of the studies had multiple end-points, those that occurred at the lowest doses were selected for modelling.

(a) Data from studies with purified fumonisin B_1

In long-term feeding studies, purified FB_1 caused both liver and kidney tumours in rodents (NTP, 2001). As the renal tumours occurred at lower doses, this carcinogenic end-point was chosen for BMD modelling. In addition, the renal toxicity that, in part, served as the basis for the NOAEL in the previous evaluation was modelled (Table 22).

Howard et al. (2002) conducted a 28-day study with B6C3F1 female mice. In addition to reporting effects of fumonisin on various measures of sphingolipid metabolism, the incidence of lesions in the liver was also recorded. As these were the lesions occurring at the lowest doses, apoptosis and hypertrophy were selected for BMD modelling (Table 23).

Table 22. Dose–response relationship for renal toxicity and tumours in male Fischer 344N rats fed diets containing purified fumonisin B_1 for 2 years, from NTP (2001)

Dose of FB_1 (mg/kg bw per day)	No. of animals showing signs of renal toxicity and tumours		
	Cytotoxic or regenerative lesions	Atypical tubule hyperplasia	Renal tumours
Untreated controls	0/42	0/48	0/48
0.22	0/40	0/40	0/40
0.67	23/33	0/48	0/48
2.2	42/42	4/48	10/48
6.6	43/43	9/48	16/48

Table 23. Incidence of hepatic lesions in female B6C3F1 mice fed diets containing purified fumonisin B_1 for 28 days, from Howard et al. (2002)

Dose of FB_1 (mg/kg bw per day)	Apoptosis	Hypertrophy
Untreated controls	0/16	0/16
2.2	0/8	0/8
11.5	8/8	5/8
22.9	8/8	8/8

Bondy et al. (2010) conducted a 2-year study with mice. The liver was found to be the primary target organ, with a variety of pathological lesions reported. Megalocytic hepatocytes appeared to be the lesion that occurred at the lowest doses (Table 24). A more commonly noted pathological lesion, apoptosis, was selected for modelling as well (Table 25). As there appeared to be little difference in results, transgenic (+/+) and wild-type (+/–) mice were pooled for BMD estimation.

(b) Data from studies with culture material using fumonisin B_1 as a marker for exposure to Fusarium

Riley & Voss (2006) conducted a small study with male rats that examined the relationship of exposure to culture material and renal toxicity as a function of dose and duration of exposure, with exposure periods of 1, 3, 5 and 10 days. Because the group sizes were small ($n = 3$) and because the outcomes at 5 and 10 days were very similar, the results at 5 and 10 days were pooled for BMD modelling (Table 26). As total concentrations were reported in the paper, FB_1 concentrations were calculated using the ratios also provided in the report.

In order to estimate a BMD from the Voss et al. (2011) study, individual subject data for renal pathology were obtained from the study authors. Because a score of 1 is not clearly treatment related and because the group sizes are small ($n = 5$), a pathology score of 2 was used as the benchmark response (BMR). This

Table 24. Individual pathology scores for megalocytic hepatocytes, from Bondy et al. (2010)

Group	Average dose (mg/kg bw per day)	Individual scores (n = 8–10)
Controls		
- transgenic (+/+)	0	0 0 0 0 0 0 0 0 0 0
- wild type (+/–)	0	0 0 0 0 0 0 0 0 0 0
5 mg/kg		
- transgenic (+/+)	0.4	0 0 0 0 0 0 0 1 0 2 0
- wild type (+/–)	0.4	0 1 0 0 0 0 0 0 1 0
50 mg/kg		
- transgenic (+/+)	4	1 0 0 2 1 0 0 1 0 1
- wild type (+/–)	4	2 2 2 0 0 0 0 0 0 0
150 mg/kg		
- transgenic (+/+)	12	5 5 5 5 4 3 3 5 5 5
- wild type (+/–)	12	5 5 5 5 5 5 5 5

Table 25. Individual pathology scores for apoptosis, from Bondy et al. (2010)

Group	Average dose (mg/kg bw per day)	Individual scores (n = 8–10)
Controls		
- transgenic (+/+)	0	1 1 1 1 1 1 1 1 1 1
- wild type (+/–)	0	1 1 1 1 1 1 1 1 1 1
5 mg/kg		
- transgenic (+/+)	0.4	0 0 0 0 0 0 1 0 0 0
- wild type (+/–)	0.4	0 0 0 0 0 0 0 0 0 0
50 mg/kg		
- transgenic (+/+)	4	4 3 1 4 4 2 2 2 1 3
- wild type (+/–)	4	4 3 4 3 3 5 1 4 3
150 mg/kg		
- transgenic (+/+)	12	5 5 5 5 4 3 3 5 5 5
- wild type (+/–)	12	5 5 5 5 5 5 5 5

study employed two different batches of culture material that underwent different processing steps, which resulted in a range of dietary exposures. Two groups of five animals were sacrificed and examined after 3 and 8 weeks. Doses based on FB_1 content and individual pathology scores are listed in Tables 27 and 28.

Table 26. Incidence of renal lesions in Sprague-Dawley rats fed diets containing culture material using fumonisin B_1 as a marker for 5–10 days, from Riley & Voss (2006)

Dose of FB_1 (mg/kg bw per day)	Incidence of renal toxicity
0.07 (controls)	0/6
0.9	3/6
5.7	6/6

Table 27. Individual renal pathology scores, week 3, from Voss et al. (2011)

Diet	Average dose (µg/kg bw per day)	Individual scores (n = 5)
Controls		
- Uncooked	0	0 0 0 0 1
- Extruded	0	0 0 0 0 0
Fermented grits, low FB_1		
- Batch 1	468	3 2 3 2 2
- Batch 1E	143	0 1 2 2 1
- Batch 1EG	34	0 0 1 0 0
Fermented grits, high FB_1		
- Batch 2	2419	3 3 3 3 3
- Batch 2E	923	3 3 3 3 3
- Batch 2EG	298	2 0 1 1 1

Table 28. Individual renal pathology scores, week 8, from Voss et al. (2011)

Diet	Average dose (µg/kg bw per day)	Individual scores (n = 5)
Controls		
- Uncooked	0	0 0 0 0 0
- Extruded	0	0 0 0 0 0
Fermented grits, low FB_1		
- Batch 1	354	2 2 2 0 2
- Batch 1E	103	2 0 1 0 0
- Batch 1EG	25	0 0 0 0 0
Fermented grits, high FB_1		
- Batch 2	1804	3 3 3 3 3
- Batch 2E	698	0 3 3 3 0
- Batch 2EG	222	0 0 3 0 3

9.3 Benchmark dose estimates

9.3.1 Studies with purified fumonisin B_1

(a) *Two-year study, renal cytotoxicity in male rats*

Six of the nine dichotomous models fit the data very well, whereas three others that fit poorly or not at all were excluded. Of the former six models, the lowest $BMDL_{10}$ of 286 µg/kg bw per day was obtained with the log-probit model (Table 29 and Figure 10).

(b) *Two-year study, renal tumours in male rats*

As seven of the nine models fit the data reasonably well, the two others were excluded. The lowest $BMDL_{10}$ of 1108 µg/kg bw per day was obtained with the log-logistic model (Table 30 and Figure 11).

(c) *Twenty-eight-day study, hepatic apoptosis in female mice*

Two of the nine models were excluded based on goodness of fit. Of the remaining models, the lowest $BMDL_{10}$ of 944 µg/kg bw per day was obtained with the multistage model (Table 31 and Figure 12).

(d) *Twenty-eight-day study, hepatic hypertrophy in female mice*

None of the nine models were excluded based on goodness of fit. The lowest $BMDL_{10}$ of 673 µg/kg bw per day was obtained with the quantal-linear model and the identical multistage cancer model (Table 32 and Figure 13).

(e) *Six-month study, megalocytic hepatocytes in male mice*

As occurrence appeared to be dose related, a pathology score of 1 was selected as the end-point to be modelled. The log-probit and multistage models were excluded for that reason. Of the seven other models, the lowest $BMDL_{10}$ of 165 µg/kg bw per day was obtained with the log-logistic model (Table 33 and Figure 14).

(f) *Six-month study, apoptosis in male mice*

Seven of the models fit the data quite well, whereas the other two that did not (multistage cancer and quantal-linear) were excluded. Of the seven remaining models, the lowest $BMDL_{10}$ of 463 µg/kg bw per day was obtained with the multistage model (Table 34 and Figure 15).

9.3.2 Studies using fumonisin B_1 as a marker for Fusarium

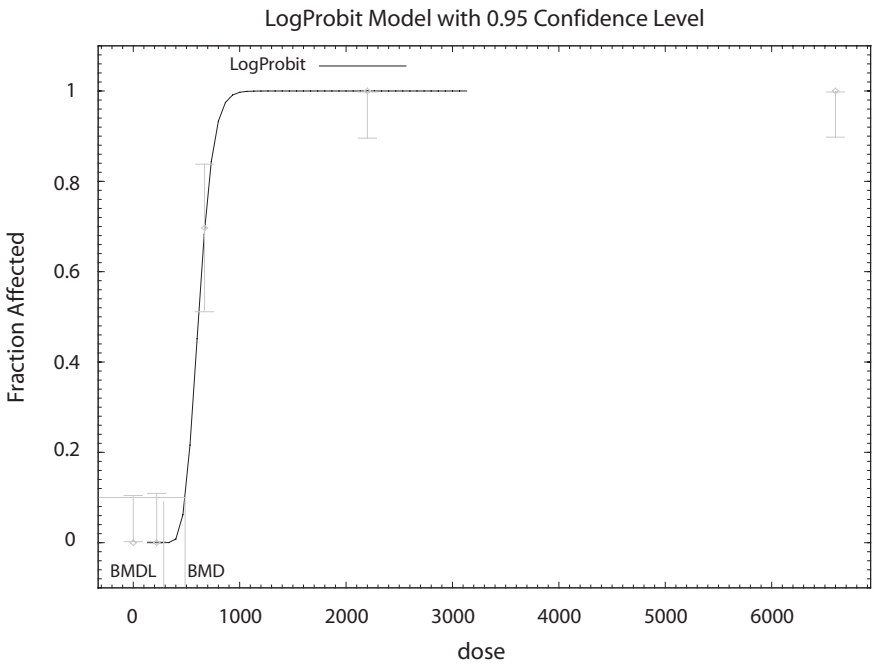
(a) *Five or 10 days of exposure, renal toxicity in male rats*

Because the data contained only three dose levels, the multistage and multistage cancer models were not used, as both have three parameters or more. The remaining models fit the data well. The lowest $BMDL_{10}$ of 62 µg/kg bw per day was obtained with the quantal-linear model (Table 35 and Figure 16).

Table 29. BMD_{10} and $BMDL_{10}$ for renal cytotoxicity in male rats, based on NTP (2001)

Model name	*P*-value	BMD_{10} (µg/kg bw per day)	$BMDL_{10}$ (µg/kg bw per day)
Gamma	1.00	431	295
Logistic	1.00	602	356
Log-logistic	1.00	565	306
Log-probit	1.00	487	286
Multistage	0.26	222	180
Multistage cancer	0.00	78	61
Probit	1.00	541	328
Weibull	1.00	496	313
Quantal-linear	0.00	78	61

Figure 10. BMD_{10} and $BMDL_{10}$ for renal toxicity from NTP (2001) using the log-probit model



(b) Three-week study, renal toxicity in male rats

All nine of the models fit the data reasonably well. The lowest $BMDL_{10}$ of 21 µg/kg bw per day was obtained with the quantal-linear and identical multistage cancer models (Table 36 and Figure 17).

Table 30. BMD_{10} and $BMDL_{10}$ for renal tumours, based on NTP (2001)

Model name	*P*-value	BMD_{10} (µg/kg bw per day)	$BMDL_{10}$ (µg/kg bw per day)
Gamma	0.13	1859	1218
Logistic	0.00	3478	2883
Log-logistic	0.17	1787	1108
Log-probit	0.10	2118	1692
Multistage	0.28	1603	1178
Multistage cancer	0.28	1603	1178
Probit	0.00	3200	2642
Weibull	0.13	1827	1206
Quantal-linear	0.28	1603	1178

Figure 11. BMD_{10} and $BMDL_{10}$ for renal tumours from NTP (2001) using the log-logistic model

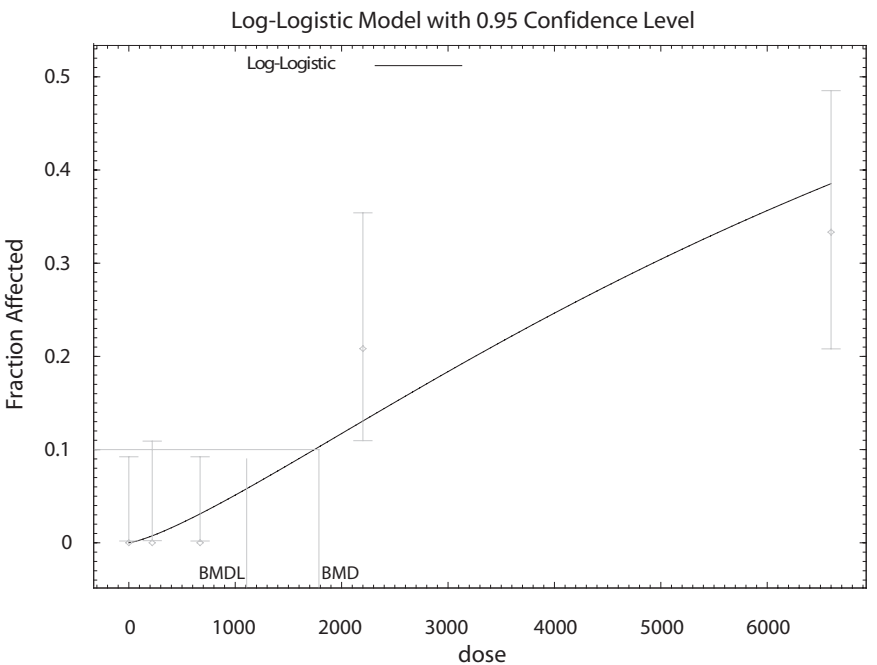


Table 31. BMD_{10} and $BMDL_{10}$ for hepatic apoptosis, based on Howard et al. (2002)

Model name	*P*-value	BMD_{10} (μg/kg bw per day)	$BMDL_{10}$ (μg/kg bw per day)
Gamma	1.00	4030	1897
Logistic	1.00	6576	2064
Log-logistic	1.00	4482	1983
Log-probit	1.00	4449	1979
Multistage	0.72	2053	944
Multistage cancer	0.16	605	339
Probit	1.00	6176	1917
Weibull	1.00	8443	1797
Quantal-linear	0.16	605	339

Figure 12. BMD_{10} and $BMDL_{10}$ for hepatic apoptosis from Howard et al. (2002) using the multistage model

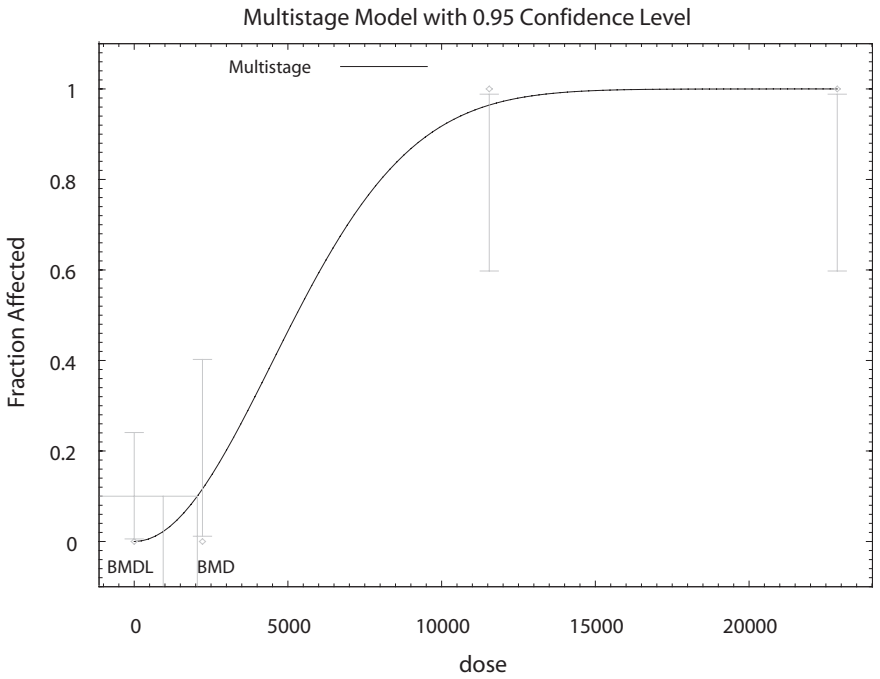


Table 32. BMD_{10} and $BMDL_{10}$ for hepatic hypertrophy, based on Howard et al. (2002)

Model name	*P*-value	BMD_{10} (µg/kg bw per day)	$BMDL_{10}$ (µg/kg bw per day)
Gamma	1.00	7 776	2 243
Logistic	1.00	10 260	3 939
Log-logistic	1.00	9 937	2 765
Log-probit	1.00	9 413	2 595
Multistage	0.93	3 682	1 414
Multistage cancer	1.00	1 109	673
Probit	1.00	9 050	3 449
Weibull	0.40	8 919	2 085
Quantal-linear	1.00	1 109	673

Figure 13. BMD_{10} and $BMDL_{10}$ for hepatic hypertrophy from Howard et al. (2002) using the quantal-linear model

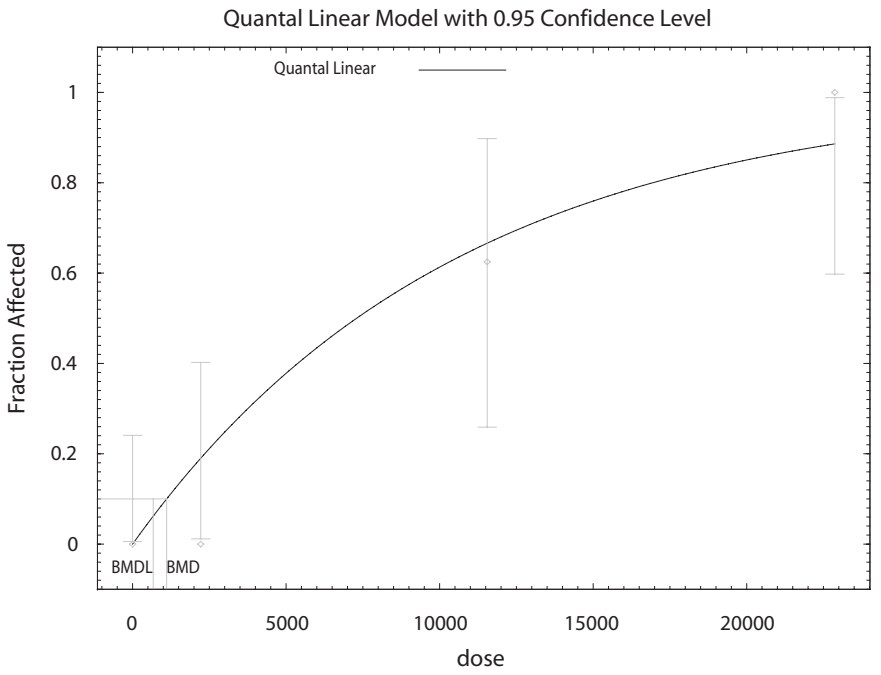


Table 33. BMD_{10} and $BMDL_{10}$ for megalocytic hepatocytes, based on Bondy et al. (2010)

Model name	*P*-value	BMD_{10} (µg/kg bw per day)	$BMDL_{10}$ (µg/kg bw per day)
Gamma	0.08	538	366
Logistic	0.15	1 675	1 178
Log-logistic	0.13	284	165
Log-probit	0.03	2 381	880
Multistage	0.04	733	372
Multistage cancer	0.08	538	366
Probit	0.15	1 613	1 176
Weibull	0.08	538	366
Quantal-linear	0.08	777	538

Figure 14. BMD_{10} and $BMDL_{10}$ for megalocytic hepatocytes from Bondy et al. (2010) using the log-logistic model

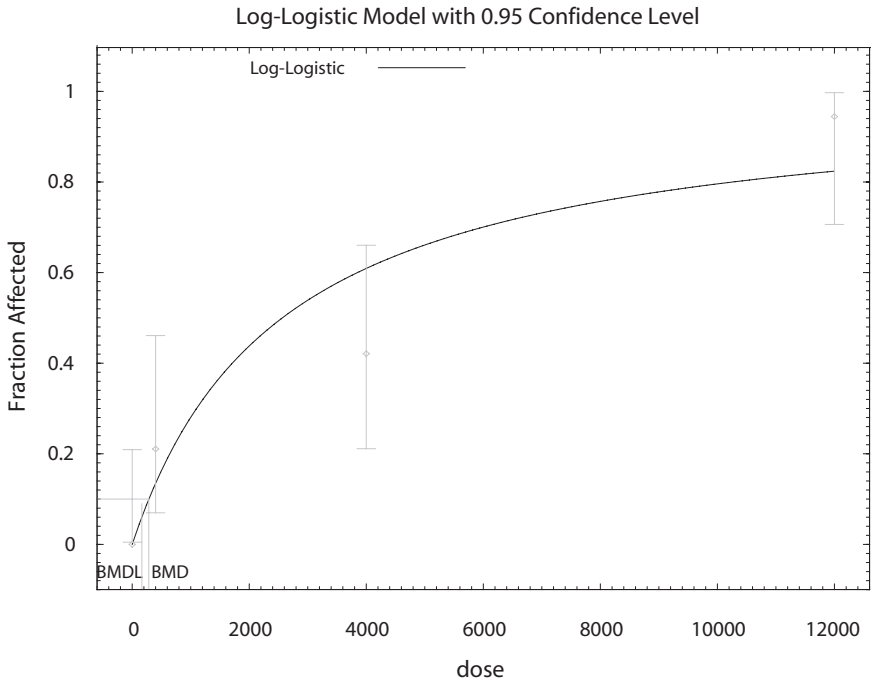


Table 34. BMD_{10} and $BMDL_{10}$ for hepatic apoptosis based on Bondy et al. (2010)

Model name	*P*-value	BMD_{10} (μg/kg bw per day)	$BMDL_{10}$ (μg/kg bw per day)
Gamma	1.00	2108	519
Logistic	1.00	3342	1216
Log-logistic	1.00	3155	560
Log-probit	1.00	2373	510
Multistage	0.95	969	463
Multistage cancer	0.30	274	184
Probit	1.00	2778	1061
Weibull	1.00	2306	528
Quantal-linear	0.30	274	184

Figure 15. BMD_{10} and $BMDL_{10}$ for hepatic apoptosis from Bondy et al. (2010) using the multistage model

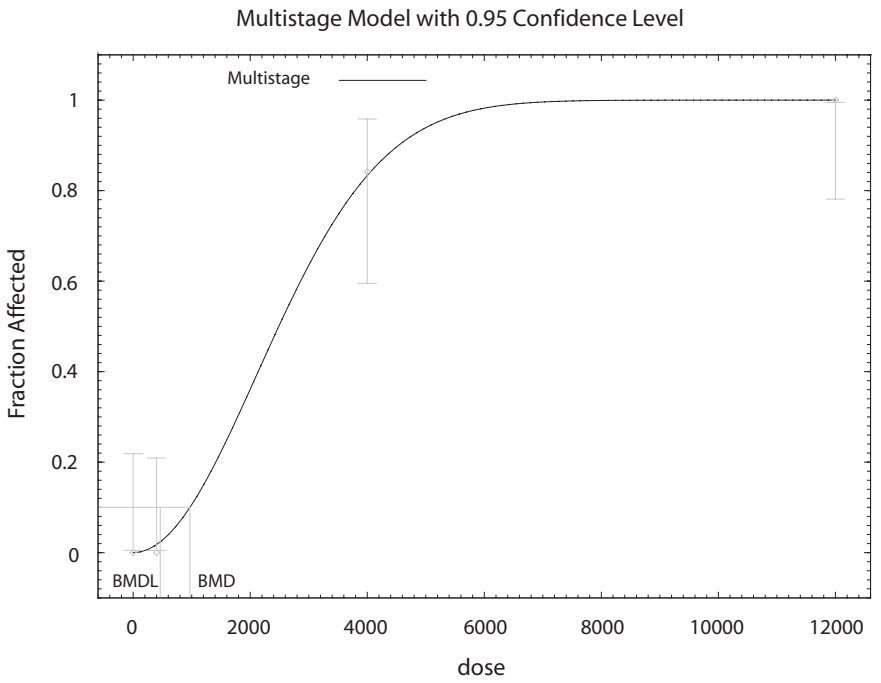


Table 35. BMD_{10} and $BMDL_{10}$ for renal pathology after 5 or 10 days of exposure, based on Riley & Voss (2006)

Model name	*P*-value	BMD_{10} (μg/kg bw per day)	$BMDL_{10}$ (μg/kg bw per day)
Gamma	1.00	567	70
Logistic	1.00	778	208
Log-logistic	1.00	700	66
Log-probit	1.00	570	114
Multistage	1.00	692	193
Multistage cancer	1.00	594	70
Quantal-linear	0.81	134	62

Figure 16. BMD_{10} and $BMDL_{10}$ for renal toxicity from Riley & Voss (2006) using the quantal-linear model

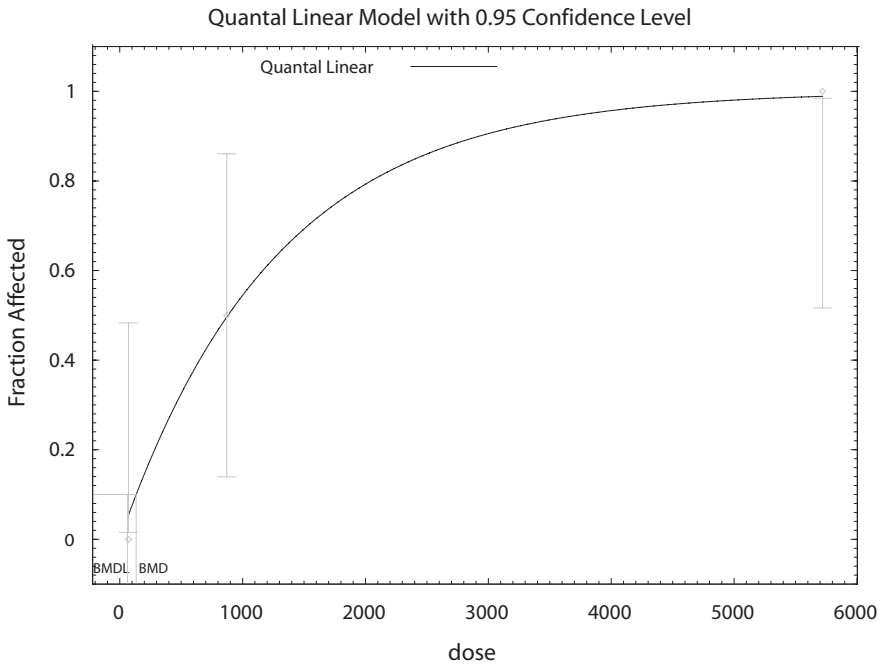
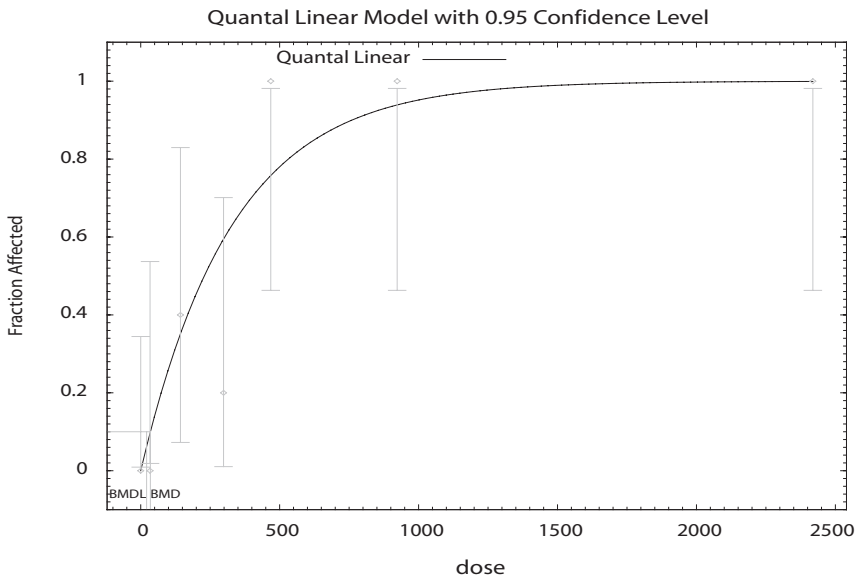


Table 36. BMD_{10} and $BMDL_{10}$ for renal pathology after 3 weeks of exposure based on Voss et al. (2011)

Model name	*P*-value	BMD_{10} (µg/kg bw per day)	$BMDL_{10}$ (µg/kg bw per day)
Gamma	0.39	96	26
Logistic	0.29	139	79
Log-logistic	0.30	102	32
Log-probit	0.33	100	42
Multistage	0.42	93	27
Multistage cancer	0.45	35	21
Probit	0.33	127	73
Weibull	0.40	101	27
Quantal-linear	0.45	35	21

Figure 17. BMD_{10} and $BMDL_{10}$ for renal toxicity at 3 weeks from Voss et al. (2011) using the quantal-linear model



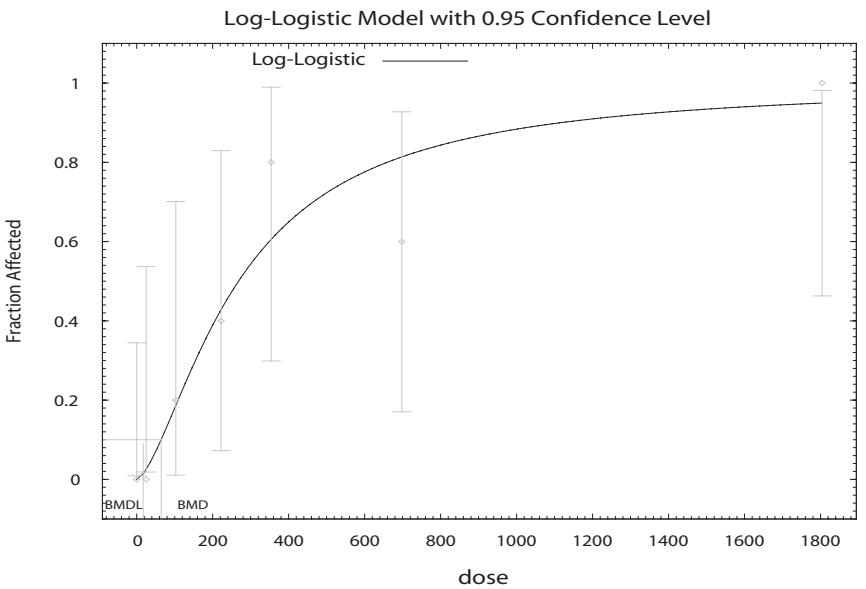
(c) Eight-week study, renal toxicity in male rats

The logistic and probit models clearly did not fit the data as well as the other seven models. The lowest $BMDL_{10}$ of 17 µg/kg bw per day was obtained with the log-logistic model (Table 37 and Figure 18).

Table 37. BMD_{10} and $BMDL_{10}$ for renal pathology after 8 weeks of exposure, based on Voss et al. (2011)

Model name	*P*-value	BMD_{10} (µg/kg bw per day)	$BMDL_{10}$ (µg/kg bw per day)
Gamma	0.73	51	29
Logistic	0.23	138	86
Log-logistic	0.75	65	17
Log-probit	0.83	76	47
Multistage	0.84	47	29
Multistage cancer	0.84	47	29
Probit	0.24	134	87
Weibull	0.73	48	29
Quantal-linear	0.84	47	29

Figure 18. BMD_{10} and $BMDL_{10}$ for renal toxicity at 3 weeks from Voss et al. (2011) using the log-logistic model



10. COMMENTS

10.1 Absorption, distribution, metabolism and excretion

In the previous evaluation, it was concluded that FB_1 and FB_2 were poorly absorbed from the digestive tract and mostly eliminated unchanged in the faeces. The small amount that was absorbed (<4%) was rapidly distributed and eliminated. Liver and kidney contained the highest levels of the absorbed material. It was also concluded that FB_1 does not cross the placenta, and, even after dosing of high levels to cows, no FB_1 was detected in the milk.

Since then, studies show that the bioavailability of FB_1 may be much greater than that of FB_2 and FB_3. Also, recent studies performed in pregnant mice indicate that FB_1 can cross the placental barrier. In other studies, it was shown that FB_1 did not cross the blood–brain barrier. Bioavailabilities of hidden or bound fumonisins have not been studied.

In the previous evaluation, there was no evidence that FB_1 was metabolized in vitro or in vivo by animal tissues, although hydrolysis by intestinal flora has been demonstrated. Since then, HFB_1 and $PHFB_1$ have been reported in low concentrations in liver (<1 µg/kg) and many other tissues in pigs, but their origin is unclear. It was also shown that FB_1, FB_2 and FB_3 do not carry over in significant amounts from feed to animal products, either as parent compounds or as their hydrolysis products.

The previously described putative mode of action of fumonisins leading to toxicity—i.e. disruption of lipid metabolism—is supported by many recent studies. A new group of sphingoid bases has been discovered and shown to accumulate in livers of FB_1-treated mice. As indicated in the previous evaluation, animals exposed to FB_1 accumulate high levels of sphingoid bases and sphingoid base 1-phosphates in blood and tissues, often at levels of exposure that do not cause overt toxicity. In experimental animal studies, the extent of disruption of sphingolipid metabolism correlates well with toxicity and has been used successfully to demonstrate structure–activity relationships, tissue specificity, strain/sex susceptibility and the efficacy of intervention strategies in poultry, fish, rats, mice, pigs and horses. Hence, the elevation in levels of the sphingoid bases sphinganine and sphingosine and the ratio of sphinganine to sphingosine in serum and urine has been validated in numerous animal studies as biomarkers for fumonisin disruption of sphingolipid metabolism. However, the level of accumulation of bioactive lipid metabolites or depletion of lipids that constitutes a quantitative measure of adverse physiological effects is not known. Therefore, the Committee concluded that although disruption of lipid metabolism is an initial response to fumonisin exposure, it could not be used as a toxicological end-point for the final evaluation.

In humans, levels of the sphingoid bases sphinganine and sphingosine and their ratio sphinganine:sphingosine in serum, urine and buccal cells have been studied as potential biomarkers. The conflicting results of several studies on sphingoid bases indicate that levels of these bases and their ratio are not valid biomarkers of human fumonisin exposure. Other suggested biomarkers of exposure in humans were fumonisin levels in hair or urine. Fumonisin levels in hair were

measured in one human study, but they were not compared with actual dietary fumonisin exposure, so currently this is also not a validated biomarker of fumonisin exposure. Urinary FB_1 level has emerged as a candidate for a human biomarker of fumonisin exposure, for several reasons. In pilot studies conducted in different parts of the world, urinary FB_1 levels correlated with imputed and actual dietary fumonisin levels. When an intervention was applied in rural South Africa to reduce fumonisins in maize porridge, levels of urinary FB_1 decreased in a study population whose baseline and post-intervention biomarker levels were measured.

10.2 Toxicological data

At the time of the previous evaluation, only a few acute studies using FB_1 were available. From these, the Committee concluded that fumonisins were not acutely toxic. Since then, a study in male rats indicated that the single oral lethal dose of FB_1 would be greater than 46.4 mg/kg bw. Other studies showed that acute effects of FB_1 in rats are similar to those of longer exposures; that is, disruption of sphingolipid metabolism is an early response. Also, increased apoptosis of centrilobular hepatocytes and tubular epithelia in the outer medulla in kidney is an early indicator of FB_1 toxicity. In pigs, early signs of PPO were induced with a single oral administration of 5 mg/kg bw of pure FB_1. One gavage study in male rats indicated that the administration of a single dose of pure FB_1 at 0.005 mg/kg bw caused an increase in apoptotic cells in the liver. This dose is orders of magnitude lower than the doses found to increase apoptosis in studies of longer duration (e.g. >0.67 mg/kg bw per day in a 2-year study; NTP, 2001). This, together with a lack of clarity in other aspects of the study, led the Committee to consider it inappropriate to use this value in the evaluation without further studies supporting this result.

Since the last evaluation, there have been only two feeding studies examining dose–response relationships in mice using pure FB_1. The first study examined hepatotoxicity and found increased apoptosis and hypertrophy in centrilobular hepatocytes and other microscopic changes indicative of liver toxicity at 11.5 mg/kg bw per day in a 28-day study in female mice. In the second study, male mice (10 mice per group), heterozygous (p53+/–) or wild type (p53+/+), were fed diets containing pure FB_1 at 0, 5, 50 or 150 mg/kg diet, reported to be equal to 0, 0.4, 4 and 12 mg/kg bw per day, for 6 months. Light microscopic evaluation of the livers revealed that increased incidence of megalocytic hepatocytes was a dose-dependent effect observed at all dose levels greater than or equal to 0.4 mg/kg bw per day in both the wild-type and transgenic mice. Multifocal necrosis and increased apoptosis in centrilobular hepatocytes were treatment-related effects in both the medium- and high-dose (≥4 mg/kg bw per day) wild-type and transgenic treatment groups. The incidences of megalocytic hepatocytes, multifocal necrosis and increased apoptosis in centrilobular hepatocytes were not significantly different between the wild-type and transgenic treatment groups. The Committee concluded that both studies were suitable to be used in this evaluation.

Since the last evaluation, there have been three feeding studies using pure FB_1 in male rats. The Committee concluded that these studies were not suitable to be used in this evaluation, as one was conducted with a single high dose (>15 mg/kg bw), and effects were seen only at high doses (>10 mg/kg bw per day) in the

other studies. Also, the measured end-points (altered lipid metabolism and reduced body weight gain) were not considered critical to the evaluation.

Two studies conducted in mice using diets prepared with *F. verticillioides* culture material were evaluated by the Committee, and it was concluded that they were not suitable to be used in this evaluation, as one was conducted with a single high dose (>45 mg/kg bw), and effects (altered lipid metabolism) were seen only at high doses (>7.5 mg/kg bw per day) in the other study.

There have been several recent studies conducted in male rats consuming diets prepared with *F. verticillioides* culture material. In two of these studies, renal toxicity was observed (primary effect increased tubular apoptosis in the outer medulla) at FB_1 doses lower than for pure FB_1, as seen in the previous evaluation. The Committee concluded that these studies were suitable for comparing the renal toxicity of FB_1 derived using *F. verticillioides* culture material diets with that of pure FB_1 in the diet. There were also a few studies conducted in rabbits and pigs using culture material in the diet. One study in rabbits showed dose–response effects on kidney and liver parameters (necrosis and other lesions). As the fumonisin content of the diets was uncertain, the Committee concluded that this precluded the use of the rabbit and pig studies for this evaluation.

There have been no new long-term feeding studies reported since the previous evaluation.

Previously, the Committee concluded that "neither fumonisin B_1 nor any other fumonisin was shown unequivocally to be genotoxic", based on a small number of studies in vitro and a single study in vivo. In studies reviewed for the present evaluation, no direct adducts of fumonisin with DNA have been found. The new data that are available generally support the previous conclusion that FB_1 is not mutagenic. The formation of 8-OH-dG adducts observed in new data that are available is indicative of oxidative damage caused by FB_1. This indicates that FB_1 induces effects, such as the formation of reactive oxygen species, that can lead to DNA damage.

In the previous evaluation, it was concluded that reproductive effects (embryotoxicity, skeletal and soft tissue malformations) were secondary to maternal toxicity and that ^{14}C-labelled FB_1 did not cross the placenta when administered orally. Since then, a mouse model has been used to demonstrate that FB_1 can induce NTDs in vivo when administered (gestation days 7.5 and 8.5) either by gavage or by intraperitoneal exposure (dose range around 20 mg/kg bw per day). ^{14}C-labelled FB_1 was shown to enter the embryo when administered intraperitoneally early in organogenesis (gestation day 10.5), and this was confirmed by elevation of sphinganine levels in embryos. Maternal liver toxicity was not assessed; however, the gavage dose of FB_1 used in this study was similar to that known to induce maternal toxicity in CD-1 mice (25 mg/kg bw per day). Moreover, NTDs were induced in one feeding study conducted in mice, but a follow-up study including higher doses was unable to confirm these results. Studies in rabbits and pigs have shown significant effects on reproductive performance in animals consuming FB_1 in the diet at doses as low as 0.12 mg/kg bw per day; however, the Committee concluded that the

uncertainty in the analysis of the diets prepared using *F. verticillioides* culture material precluded these studies from being used in this evaluation.

Two independent studies showed that pure HFB_1 did not cause maternal liver or kidney toxicity or embryotoxicity or induce NTDs in rats or mice at doses much higher than those at which FB_1 induced embryotoxicity in rats and NTDs in mice. This indicates that HFB_1 is not a risk factor for either embryotoxicity or NTDs.

Depression of specific and nonspecific immune responses and altered cytokine profiles were seen after exposure to either pure FB_1 or *F. verticillioides* culture in a variety of studies, mostly performed in pigs. In a pig study using *F. verticillioides* culture material in the diet, males were more sensitive than females to the immunomodulatory effects of FB_1; after subcutaneous exposure of mice to FB_1, females appeared more sensitive. In the last study, an immunostimulatory effect was found. There was one oral repeated-dose pig study performed with culture material derived from *F. moniliforme* (now named *F. verticillioides*), in which the authors concluded that there were no effects on immune response at doses up to 4 mg/kg bw per day; however, not all data were reported, and this study was therefore considered not suitable for the evaluation. Overall, the Committee considered the effects on the immune system to be relevant, as they were observed at low oral doses (i.e. 0.3 mg/kg bw per day for FB_1 as marker for culture material); however, they could not be used in the evaluation, as the relevant studies were performed using single doses.

Intracerebroventricular administration of FB_1 in mice caused severe neurodegenerative and behavioural changes that were not seen when mice were dosed subcutaneously. Dosing by subcutaneous administration caused slight elevation in sphinganine in the brain, but no neurodegenerative changes, at doses of FB_1 causing large elevations in ALT and AST activities and other signs of marked toxicity. The Committee concluded that these sphinganine changes could not be definitively attributed to FB_1 crossing the blood–brain barrier. In pigs fed diets containing pure FB_1, changes in acetylcholinesterase activity in several brain regions were observed. The Committee concluded that, owing to the poorly defined dietary doses, discrepancies in the reported doses and lack of a clear dose–response relationship, these effects on acetylcholinesterase activity could not be attributed to FB_1 exposure. Overall, based on these studies, the Committee concluded that it was unlikely that fumonisins could cross the blood–brain barrier and induce neurotoxic effects in the brain.

10.2.1 Co-exposure to fumonisins and other mycotoxins

In a large number of in vitro and in vivo studies, the combined effects of fumonisins and other mycotoxins have been investigated. The studies show inconclusive and sometimes contradictory results. The effects of simultaneous exposure tend to be at most additive. In some in vitro and in vivo studies, the authors suggested that synergism or antagonism may occur, but often only single doses of each individual mycotoxin were used, and the Committee concluded that these study designs were inadequate to detect synergism. Overall, the available toxicity data on co-exposure were inadequate for their use by the Committee in its

evaluation. The Committee concluded that because the fumonisins known to date do not share a similar mode of action with any other mycotoxins, it was unlikely that simple additive effects based on this mode of action would occur, although it recognized that other forms of interaction may occur. The Committee noted that co-exposure to AFB_1, a compound with known genotoxic properties, and fumonisins, which have the potential to induce regenerative cell proliferation, would be of concern.

In the previous evaluation, it was concluded that *F. verticillioides* culture material containing FB_1 and pure FB_1 were nephrotoxic in male rats. However, in the present evaluation, the nephrotoxicity of culture material was much greater than the nephrotoxicity of pure FB_1. For example, the dose of purified FB_1 that was required to cause renal tubular apoptosis in male rats was about 8 times higher than the dose of culture material containing FB_1 causing the same effect. Analysis of the culture material diets found that in addition to FB_1, FB_2, FB_3 and fumonisin–fructose adducts were present. Other fumonisins (e.g. B series, C series, A series) and unknown metabolites produced by *F. verticillioides* were also likely present in the culture material diet. Thermal processing of culture material used in diets produced fumonisin degradation products and promoted FB_1 binding to protein and carbohydrates. Thus, the Committee concluded that although FB_1 does not completely account for the total toxic potential of fumonisins and other metabolites of *Fusarium* in the diet, it is a suitable marker for dietary exposure to mycotoxins in food and feed contaminated by *Fusarium*.

10.3 Observations in humans

All of the epidemiological studies conducted since the previous evaluation that have investigated the link between fumonisin exposure and oesophageal cancer are ecological studies: whole populations were characterized (e.g. a population with high oesophageal cancer incidence) rather than individuals, as in cohort or case–control studies, and maize samples consumed by those populations were tested for fumonisin levels. Nonetheless, the weight of evidence from these studies and the studies evaluated by the fifty-sixth meeting of the Committee is suggestive of an association between fumonisin exposure and oesophageal cancer. The association has been observed in multiple populations worldwide—the studies evaluated in this report were from China, the Islamic Republic of Iran and South Africa—although these populations have differing levels of other risk factors for oesophageal cancer (e.g. tobacco smoking and alcohol consumption). A dose–response relationship has not yet been established, nor has a toxicological mechanism been elucidated. To date, no study has shown that co-occurrence of fumonisin and aflatoxin exposures resulted in increased oesophageal cancer risk compared with fumonisin exposure alone.

Since the previous evaluation, there has been one epidemiological study on potential associations between fumonisin exposure and HIV-related mortality, one study on childhood stunting and one study on NTD incidence in human babies. As the HIV study did not include measurements of fumonisin levels in food or fumonisin exposure in humans, the Committee concluded that this study alone was

insufficient to support an association between fumonisin exposure and HIV-related mortality.

The study investigating the link between fumonisin exposure and childhood stunting in the United Republic of Tanzania indicated that infants whose estimated fumonisin exposure exceeded the PMTDI of 2 µg/kg bw established at the fifty-sixth meeting of the Committee were significantly shorter and weighed less than those whose exposure was below this PMTDI. These results are congruent with toxicological studies in animals in which fumonisin exposure was associated with reduced weight gain and feed conversion efficiency.

The study of NTD incidence among Mexican Americans on the Texas–Mexico border, combined with toxicological and earlier epidemiological studies, indicates that fumonisin exposure in pregnant women may be a contributing factor to increased NTD risk in their babies.

10.4 Analytical methods

The Committee reviewed the screening and quantitative methods for the determination of FB_1, FB_2 and FB_3 in various foods made available after the fifty-sixth meeting. It was noted that several new immunoassays and antibodies have been developed, and many manufacturers continue to offer test kits based on ELISA or immunochromatographic (lateral flow) devices. Immunoassays are inexpensive, rapid, portable and suitable for routine screening of samples, but have limitations in selectivity and reproducibility. Therefore, results obtained with immunoassays need to be confirmed with quantitative reference methods.

For a quantitative assay, the use of LC with fluorescence detection after *o*-phthaldialdehyde derivatization remains the method of choice and continues to be extensively used. Recent improvements in this method include automation of the online post-column derivatization with *o*-phthaldialdehyde.

Significant advances in fumonisin analysis have been achieved by the application of LC methods coupled to MS detection systems, as well as the use of MS-based methods for the determination of multiple mycotoxins, including fumonisin. LC-MS/MS methods are now available for the determination of some fumonisins in a large number of matrices. Despite the increasing use of LC-MS or LC-MS/MS for multiple mycotoxin analysis, only one interlaboratory validation study is available for FB_1 and FB_2 in food.

Limitations to be considered for all analytical methods are the lack of suitable reference materials for method validation and the unavailability of standards, particularly for the hydrolysed fumonisins (HFB_1, HFB_2, HFB_3) and FB_6, as well as inadequate knowledge about reactions leading to hidden and bound fumonisins.

Most of the currently used analytical methods for fumonisins are unable to detect hidden or bound fumonisins in the matrix. Further studies are required to elaborate more appropriate methodologies for their determination. The most promising methods for hidden and bound fumonisin quantification include enzymatic or acid digestion, followed by alkaline hydrolysis.

10.5 Sampling protocols

The fifty-sixth meeting of the Committee noted that the sampling stage of mycotoxin analysis can represent the greatest contribution to the overall variance of the results. Specific sampling protocols, such as the one provided by the European Commission, which is used worldwide for control, do not cover all sampling necessities. Efforts have been devoted to improving the reliability of the food-grade maize sampling. At the Fourth Session of CCCF in 2010 (FAO/WHO, 2010b), sampling plans for whole (shelled) maize, corn on the cob and maize products such as maize flour, meal, grits and processed maize flour were proposed. For maize products, it was assumed that sampling variance for these commodities was similar to that associated with aflatoxin in comminuted feeds. However, recent data reviewed for this evaluation showed that the fumonisin distribution in foods and feeds is close to normal. The Committee concluded that further investigations of fumonisin distribution in different foods and feeds are needed to improve the sampling protocols for fumonisins.

10.6 Effects of processing

The effects of various processing procedures on the levels of FB_1, FB_2 and FB_3 contamination in cereals were reviewed during the fifty-sixth meeting of the Committee. New studies on extraction characteristics of fumonisins provide a better insight into the fate of fumonisins during sorting, cleaning, thermal processing (including extrusion), milling, fermentation and alkaline treatment.

Reduction of fumonisin levels during sorting and cleaning is dependent on the initial contamination level. The reduction of fumonisins in the wet milling process is due in part to the solubility of the toxins in the steep water. Further studies are required to analyse bound fumonisins during this process as well as to determine the fate of fumonisins and their reactions in heated food. Toxin distribution in dry milled products is dependent on the milling strategies in the plants, and the general distribution pattern is similar for different fractions. Alkaline treatment produces hydrolysed forms of fumonisins that could also be involved in binding with different components of the food matrix.

10.7 Prevention and control

Fusarium species are predominantly considered to be field fungi; however, it has been reported that fumonisin production can occur post-harvest when storage conditions are inadequate.

Significantly lower fumonisin levels have been demonstrated in transgenic Bt maize, through reduction of insect pest damage and subsequent fungal infection, when compared with non-Bt isolines. In addition, new transgenic crops are being developed for detoxifying mycotoxins in the crops themselves. The use of antagonistic microorganisms has also received attention. Naturally occurring phenolic compounds, such as chlorophorin, vanillic acid, caffeic acid and maakianin, inhibit mould growth and reduce fumonisin production.

There has been increased interest in the use of natural pesticides to prevent damage to crops, such as the essential oils of menthol, oregano, thyme or

clove, as well as natural anti-oxidants, to reduce fumonisin production. New uses of microorganisms to bind or degrade fumonisins are also available. Lactic and propionic acid bacteria bind fumonisins under acidic conditions, with non-viable bacteria showing higher binding capacity. Most of these are recent developments, and their commercial or large-scale applicability remains to be seen.

10.8 Levels and patterns of contamination in food commodities

Information on the natural occurrence of fumonisins was drawn from data received from a number of countries (Argentina, Australia, Brazil, Canada, China, Ghana, Japan, Republic of Korea, Singapore, United Republic of Tanzania, Uruguay, USA), results submitted by member states of the EU (Austria, Belgium, Cyprus, Czech Republic, Estonia, France, Germany, Hungary, Lithuania, Luxembourg, the Netherlands, Slovakia and Spain) through EFSA, as well as surveys published in the open literature from 47 countries.

In total, data on 15 755 samples analysed for FB_1 in food were collected, and the occurrence levels of total fumonisins were reported or calculated for 17 091 food samples.

All occurrence data were classified according to the food groups used in the GEMS/Food consumption cluster diets. Occurrence levels of fumonisins (32% and 26% of the samples for FB_1 and total fumonisins, respectively) from processed and not sufficiently described foods were categorized separately and not used to estimate chronic dietary exposure. Two scenarios were considered when calculating the mean values; samples in which the concentration was below the LOQ or LOD were assumed to have a value of either zero (lower-bound estimates) or the limit itself (upper-bound estimates).

In total, data on 10 354 samples analysed for FB_1 in food were used to estimate chronic dietary exposure (43% from the Americas, 34% from Asia, 12% from Africa, 10% from Europe and 0.1% from Oceania). More than three quarters of the samples considered (83%) referred to maize. Mean FB_1 levels in maize vary widely between and within the GEMS/Food clusters (Table 38). FB_1 was also detected in "figs, dried" and "groundnuts, shelled". Upper-bound estimates for the global total mean for FB_1 in all other commodities and GEMS/Food clusters did not exceed 100 µg/kg.

In total, occurrence levels of total fumonisins for 12 392 food samples (49% from the Americas, 27% from Asia, 13% from Europe, 11% from Africa and 0.1% from Oceania) were used to assess dietary exposure. The majority of the samples (85%) were for maize. Also in the case of total fumonisins, levels in maize vary widely between and within the GEMS/Food clusters (Table 39). Lower- and upper-bound estimates for the global total mean of total fumonisins in all other commodities and clusters did not exceed 150 and 200 µg/kg, respectively.

Most of the occurrence data were for FB_1, FB_2 or FB_3, with recent studies showing a few samples naturally contaminated with 3-*epi*-FB_3, FB_4 and FB_6.

From the data analysed for foods, the ratios among FB_1, FB_2 and FB_3 are not constant and depend on the fungal species prevalent in different regions of

Table 38. Levels of fumonisin B_1 in food commodities

Commodity	No. of individual samples	% sample < LOD or LOQ	Global total[a] mean (µg/kg)		Mean across GEMS/Food clusters (lower- and upper-bound estimates) (µg/kg)	
			Lower bound	Upper bound	Minimum	Maximum
Barley	175	82	35	44	0	212
Buckwheat	95	100	0	3	0	3
Figs, dried	230	25	238	250	238	250
Groundnuts, shelled	16	81	97	105	97	105
Maize	8569	30	1237	1260	84	4323
Millet	42	86	0	8	0	8
Oats	17	88	1	10	1	10
Rice	242	96	2	31	0	100
Sorghum	17	76	35	74	0	151
Soya bean (dry)	137	53	33	34	0	84
Sweet corn, kernels	740	66	84	94	0	397
Wheat	74	80	38	48	0	221

[a] Global total lower- and upper-bound mean obtained by pooling the data across all GEMS/Food clusters.

Table 39. Levels of total fumonisins in food commodities

Commodity	No. of individual samples	Global total[a] mean (µg/kg)		Mean across GEMS/Food clusters (lower- and upper-bound estimates) (µg/kg)	
		Lower bound	Upper bound	Minimum	Maximum
Barley	123	51	102	0	340
Buckwheat	96	0	10	0	10
Maize	10 759	1651	1681	174	5921
Millet	42	0	18	0	18
Oats	26	1	23	0	200
Rice	207	1	59	0	104
Soya bean (dry)	106	6	11	0	30
Sweet corn, kernels	939	131	164	0	549
Wheat	94	0	29	0	200

[a] Global total lower- and upper-bound mean obtained by pooling the data across all GEMS/Food clusters.

the world. Fumonisin ratios in food also depend on the process to which they are subjected, sometimes showing different ratios between the free fumonisins in corn, and these ratios tend to be normally distributed.

Extractable and hidden fumonisins in three food products showed almost a normal distribution; however, further studies should be done to confirm these results in order to develop improved sampling protocols.

The Committee was requested by CCCF to evaluate the co-occurrence, in food and feed, of fumonisins with other mycotoxins. However, this evaluation could not be performed by the Committee, because only aggregated data were available. Levels of fumonisins and other mycotoxins must be available at the level of the individual analytical sample for such an assessment to be conducted.

10.9 Levels and patterns of contamination in feed

Information on the natural occurrence of fumonisins in feed materials was drawn from data received from a number of countries (Brazil, China, Japan, Norway, South Africa and Uruguay), results submitted by member states of the EU through EFSA (Belgium, Estonia, France, Hungary, Lithuania, the Netherlands and Slovakia), one commercial feed supplier and published surveys.

Data on a total of 19 631 samples of cereals intended for feed production, silage and finished feed were considered. The ratios between levels of each fumonisin to the sum of fumonisins ($FB_1 + FB_2 + FB_3$) varied for different countries, mostly due to different fungal prevalence.

The log-normal distribution provides an adequate fit for FB_1 and total fumonisins based on the data provided by Japan.

Data on 7060 samples originating from Africa (4.5%), America (13.3%), Asia (69.8%), Europe (4.5%) and Australia and New Zealand (7%) were selected for evaluation. These data were generated using similar methods of analysis as used for food. The global lower-bound and upper-bound mean values of the sum of FB_1, FB_2 and FB_3 for the more frequently contaminated feed materials were calculated (Table 40). Corn gluten meal, maize and dried grains plus dried soluble matter from distillers were the most contaminated, and the lower-bound mean values were found to be 3807 µg/kg, 1565 µg/kg and 1077 µg/kg, respectively.

10.10 Food consumption and dietary exposure assessment

Since the previous evaluation, a number of national evaluations of dietary exposure have been published. The Committee considered evaluations by Brazil, China, the EU (collectively), France, Guatemala, the Islamic Republic of Iran, Italy, the Netherlands, Portugal, the Republic of Korea, South Africa, Spain, the United Republic of Tanzania and the USA (see Table 17 in section 8.3). Unprocessed maize was often the only source of fumonisins considered, but in some of the studies, other cereals and cereal-based products were also taken into account. Most of these reports included dietary exposure estimates for FB_1 only (six studies), and total fumonisins were expressed as $FB_1 + FB_2$ and as $FB_1 + FB_2 + FB_3$ in six and seven evaluations, respectively. Most of the estimates were below 1 µg/kg bw per day for

Table 40. Levels of fumonisins (FB_1 + FB_2 + FB_3) in feed

Commodity	No. of individual samples	% sample < LOD or LOQ	Mean concentration (µg/kg)	
			Lower bound	Upper bound
Cereals	384	87	97	313
Corn gluten meal	18	—	3 807	3 807
Distillers' grains with solubles	78	23	825	883
Dried distillers' grains with solubles	185	14	1 077	1 110
Finished feed	2 353	30	691	765
Maize	1 927	24	1 565	1 625
Other feed	1 391	75	339	524
Rice	20	95	38	275
Silage	248	79	184	383
Soya and soya bean products	362	93	52	283
Wheat	88	89	28	250

the general population, but particularly high exposures to FB_1 (up to 7.6 and 33.3 µg/kg bw per day for consumers with average and high consumption, respectively) and total fumonisins (up to 10.6 and 44.8 µg/kg bw per day for consumers with average and high consumption, respectively) were registered in specific regions and population groups.

At the current meeting, the Committee prepared updated international estimates using the consumption cluster diets from GEMS/Food.

Individual data points on the concentration of the contaminant (for both FB_1 and total fumonisins) in foods from each cluster have been pooled to derive summary representative concentrations for each cluster for use in the dietary exposure calculations (see Tables 38 and 39 above). For each commodity, when concentration data were not available for a cluster, the global total lower- and upper-bound means, obtained by pooling the data across all clusters, were used to assess exposure. A standard body weight of 60 kg was used to assess exposure per kilogram body weight. Exposures estimated are mean exposures expressed in micrograms per kilogram body weight per day and are representative of chronic dietary exposures.

For the upper-bound scenario, the total dietary exposure to FB_1 was estimated to range from 0.3 µg/kg bw per day (cluster L) to 6.2 µg/kg bw per day (cluster A), and total dietary exposure to total fumonisins ranged from 0.4 µg/kg bw per day (clusters F and L) to 8.4 µg/kg bw per day (cluster A). No differences appeared under the lower-bound scenario.

In the previous evaluation, dietary exposure to FB_1 was evaluated by the Committee, assuming that all maize consumed contains FB_1 at the concentration found in 349 unprocessed maize samples from the Netherlands (1.4 mg/kg at the mean). Maize was the only source of FB_1 considered at that time. Using the then-available five regional diets from GEMS/Food, the total dietary exposure to FB_1 was estimated to range from 0.2 µg/kg bw per day in the European-type diet to 2.4 µg/kg bw per day in the African diet. The Committee noted that the international exposure estimates obtained in the present evaluation were higher than those of the previous evaluation. This is mainly due to the fact that occurrence data from only one country were used at that time. It was therefore not possible to take into account the high within-cluster variability for the levels of FB_1 in maize as shown by the currently available information.

The contribution of maize to the total exposure to FB_1 and to total fumonisins ranged from 14% (cluster E, lower-bound scenario) to 98% (cluster A, lower-bound scenario) and from 18% (cluster E, upper-bound scenario) to 99% (cluster H, lower-bound scenario), respectively. Wheat was the main contributor to FB_1 exposure in clusters E (80% and 78% in lower-bound and upper-bound scenarios, respectively) and B (50% and 52% in lower-bound and upper-bound scenarios, respectively). The Committee noted that overall exposure in these clusters is relatively low (maximum 1.1 µg/kg bw per day in cluster E) and that levels of FB_1 in wheat are based on only 74 samples, 80% of them below the LOD or LOQ. No differences appeared between the upper- and lower-bound scenarios. Only under the upper-bound scenario did wheat contribute to the exposure to total fumonisins, with percentages ranging from 1% (clusters A, H and M) to 44% (cluster D).

The Committee concluded that, based on the national and international estimates, dietary exposure to FB_1 for the general population ranges from 0.12×10^{-3} to 7.6 µg/kg bw per day at the mean, whereas the 95th percentile exposure was estimated to be up to 33.3 µg/kg bw per day. Dietary exposure to total fumonisins for the general population would range, for a consumer with average consumption, from 0.087×10^{-3} to 14.14 µg/kg bw per day, whereas for consumers with high consumption, exposure was estimated to be up to 44.8 µg/kg bw per day. Maize is still the predominant source of exposure to FB_1 and total fumonisins.

10.11 Impact assessment of implementation of Codex MLs in maize

CCCF has proposed the establishment of MLs for fumonisins (FB_1 + FB_2) in maize and maize-based products. In order to evaluate the potential effect of these MLs on chronic dietary exposure, all occurrence data on total fumonisins (as reported or calculated) were categorized into the groups for which an ML has been proposed: “corn/maize grain, unprocessed” (ML = 5000 µg/kg), “corn/maize flour/meal” (ML = 2000 µg/kg), “popcorn grain” (ML = 2000 µg/kg), “maize-based baby food” (ML = 500 µg/kg) and “maize-based breakfast cereals, snacks and chips” (ML = 1000 µg/kg). An international dietary exposure assessment for total fumonisins was performed based on these MLs. For this, all samples for which the upper-bound mean concentration of total fumonisins exceeded its ML were excluded from the calculation.

The percentages of rejected samples after implementation of the proposed MLs are presented, by food category and cluster, in Table 18 in section 8.4. MLs did not result in rejected samples in the majority of commodities and clusters. Overall, only 11% of the samples were excluded.

Rejection of samples was noted for "corn/maize flour/meal" in four clusters (from 4% to 57% rejected samples and from 39% to 89% reduction for the upper-bound mean), "corn/maize grain, unprocessed" in six clusters (from 1% to 88% rejected samples and from 7% to 70% reduction for the upper-bound mean), "maize-based breakfast cereals, snacks and chips" in one cluster (11% rejected samples and 30% reduction for the upper-bound mean) and "popcorn grain" in one cluster (17% rejected samples and 40% reduction for the upper-bound mean).

The effect of the implementation of the proposed Codex MLs on chronic dietary exposure to total fumonisins was evaluated by means of the GEMS/Food consumption cluster diets (see Table 18 in section 8.4). For the upper-bound scenario, reduction in exposure from all commodities occurred in nine clusters (from 6% to 68%), and the total dietary exposure to total fumonisins ranged from 0.3 µg/kg bw per day (cluster F) to 6.9 µg/kg bw per day (cluster H).

The Committee also evaluated the impact of a range of hypothetical MLs for the categories "corn/maize grain, unprocessed" (10 000, 7000, 5000, 2500, 1000 and 500 µg/kg) and "corn/maize flour/meal" (4000, 3000, 2000, 1000, 500 and 250 µg/kg) on the rejection of samples (see Tables 19 and 20 in section 8.4) and the chronic dietary exposure to total fumonisins (see Table 21 in section 8.4). Exposure estimates were obtained by using different combinations of MLs for the two above-mentioned food categories, whereas for the other food categories, the MLs proposed by the Codex committee were used.

No or little effect was noticed on the international exposure estimates resulting from the implementation of MLs higher than those proposed by CCCF. None of the evaluated MLs, including the one proposed by CCCF for the category "corn/maize flour/meal", produced a relevant reduction in exposure in all clusters. The exposure estimate in cluster M even increased, as a result of the rejection of samples with relatively low fumonisin levels within the GEMS/Food category "maize". MLs of 5000 and 2500 µg/kg for "corn/maize grain, unprocessed" reduced exposure estimates more than 50% in three and four clusters, respectively. The resulting exposure estimates were above 2 µg/kg bw per day in four, one and zero clusters based on hypothetical MLs for "corn/maize grain, unprocessed" at 2500, 1000 and 500 µg/kg, respectively.

10.12 Dose–response analysis

Four of the reviewed studies were identified as providing data suitable for BMD estimation. The first was a 28-day study performed with pure FB_1 in female mice, with the incidences of hepatic apoptosis and hypertrophy used as critical end-points (Howard et al., 2002). The second was a recent study (Bondy et al., 2010) of the effects of pure FB_1 on male transgenic p53+/– and corresponding wild-type mice. Incidences of two lesions were modelled from this study: megalocytic hepatocytes and apoptosis, where the pathology scores of 1 and 2 were used

as cut-off points for the megalocytic hepatocytes and apoptosis, respectively. The results for the transgenic and wild-type mice were pooled for dose–response analysis, as there were no differences in histopathological results between these strains of mice. The third study (Riley & Voss, 2006) examined the effects of 10-day oral exposure of male rats to *F. verticillioides* culture material in the diet. The results from day 5 and day 10 were pooled, as the results were similar. Renal toxicity was selected as an end-point, and this effect, as well as FB_1 levels in the kidney, reached a maximum for the high dose on day 5. The fourth study (Voss et al., 2011) examined the effects of *F. verticillioides* culture material on male rats using FB_1 as a marker. The incidence of renal toxicity as a function of dose was modelled for exposure durations of 3 and 8 weeks. In order to compare the results with those of the previous evaluation, the NTP (2001) study that served as its basis was also modelled. Two end-points were selected from this data set: renal toxicity and renal cell tumours in male rats.

Modelling was carried out using the USEPA's BMD software (BMDS version 2.1.2). The nine different dichotomous models provided by the program were fit to each of the data sets identified as critical. If exponential shape parameters were present, these were constrained to have values above 1. Those resulting in acceptable fits based on quantitative comparisons of each model were selected to derive BMD_{10} and $BMDL_{10}$ values for a BMR of 10% extra risk (see Tables 41 and 42). The lowest $BMDL_{10}$ for purified fumonisin, which came from the Bondy et al. (2010) study, was 165 µg/kg bw per day. The lowest calculated $BMDL_{10}$ for FB_1 as a marker came from the Voss et al. (2011) study and was 17 µg/kg bw per day.

11. EVALUATION

Exposure to fumonisins has been associated with a wide range of effects, which are often species and sex specific. Laboratory studies have identified the liver as the most sensitive organ in mice and the kidney as the most sensitive organ in rats.

Studies suitable for dose–response analysis have been conducted with rodents employing either purified FB_1 or *F. verticillioides* culture material containing FB_1. The latter studies typically use FB_1 as a marker for dietary exposure to the fumonisins and other metabolites of *Fusarium*. The studies employing purified FB_1 are generally better in experimental design for dose–response analysis. However, the Committee concluded that the studies with culture material were of sufficient quality to clearly indicate that other toxins produced by *F. verticillioides* either add to or potentiate the toxicity of FB_1. Although naturally contaminated corn would probably be more representative of actual human dietary exposure than either purified FB_1 or culture material, no suitable studies were identified that used naturally contaminated corn as a test material. As the implications are somewhat different, the Committee evaluated studies with purified FB_1 and *F. verticillioides* culture material separately.

For pure FB_1, the lowest identified $BMDL_{10}$ was 165 µg/kg bw per day for megalocytic hepatocytes in male mice. Using an uncertainty factor of 100 for intraspecies and interspecies variation, the Committee derived a PMTDI of 2 µg/

Table 41. Ranges of BMD_{10} and $BMDL_{10}$ values for dietary exposure to purified fumonisin B_1

End-point and study	BMD_{10} (μg/kg bw per day)	$BMDL_{10}$ (μg/kg bw per day)
Rat renal toxicity (NTP, 2001)	431–602	286–356
Rat renal cell tumours (NTP, 2001)	1603–2118	1108–1692
Mouse hepatocyte apoptosis (Howard et al., 2002)	2053–8443	944–2064
Mouse hepatocyte hypertrophy (Howard et al., 2002)	1109–10 260	673–3939
Mouse megalocytic hepatocytes (Bondy et al., 2010)	284–1675	165–1178
Mouse hepatocyte apoptosis (Bondy et al., 2010)	969–3342	463–1216

Table 42. Ranges of BMD_{10} and $BMDL_{10}$ values for dietary exposure to* Fusarium *culture material using fumonisin B_1 as a marker

End-point and study	BMD_{10} (μg/kg bw per day)	$BMDL_{10}$ (μg/kg bw per day)
Rat renal toxicity 5–10 days (Riley & Voss, 2006)	134–778	62–208
Rat renal toxicity 3 weeks (Voss et al., 2011)	35–139	21–79
Rat renal toxicity 8 weeks (Voss et al., 2011)	47–76	17–47

kg bw. As this was the same value as the previously established group PMTDI, this group PMTDI, for FB_1, FB_2 and FB_3, alone or in combination, was retained.

For culture material, the lowest identified $BMDL_{10}$ using FB_1 as a marker was 17 μg/kg bw per day for renal toxicity in male rats. The Committee chose not to establish a health-based guidance value for culture material because its composition was not well characterized and may not be representative of natural contamination.

The Committee concluded that, based on the national and international estimates, dietary exposure to FB_1 for the general population ranges from 0.12×10^{-3} to 7.6 μg/kg bw per day at the mean, whereas the 95th percentile exposure was estimated to be up to 33.3 μg/kg bw per day. Dietary exposure to total fumonisins for the general population would range, for an average consumer, from 0.087×10^{-3} to 14.14 μg/kg bw per day, whereas for consumers with high consumption, exposure was estimated to be up to 44.8 μg/kg bw per day. Maize is still the predominant source of exposure to FB_1 and total fumonisins.

Comparison of these estimates with the group PMTDI indicates that the group PMTDI is exceeded at the population level in some regions within some countries. The Committee concluded that adverse effects from fumonisin exposure may occur and that reduction of exposure to fumonisin and other toxins produced by *F. verticillioides* is highly desirable, particularly in areas of the world where maize is a major dietary staple food and where high contamination can occur.

As fumonisins do not carry over from feed to animal products in significant amounts, the occurrence of fumonisins in feed was considered not to be a human health concern.

The Committee concluded that implementation of the MLs proposed by CCCF could significantly reduce exposure (by more than 20%) to total fumonisins in six clusters (A, B, D, F, G, K). The main contribution to reduction was due to the proposed Codex ML for the category "corn/maize grain, unprocessed". The Committee noted that implementation of the proposed MLs would result in rejection of 1–88% of "corn/maize grain, unprocessed" and 4–57% of "corn/maize flour/meal" across the clusters. The Committee also noted that the national estimates of exposure to fumonisins show that the exceedance of the PMTDI occurs only in limited regions presenting high maize consumption levels and highly contaminated maize.

The Committee concluded that no or little effect on the international exposure estimates was noticed as a result of implementing MLs higher than those proposed by CCCF.

11.1 Research needs

To be able to fully assess the toxic potential of culture material or naturally contaminated food, characterization and quantification of their mycotoxin content are necessary.

To obtain a realistic representation of the effects of "real life" exposure, and in order to compare its toxic potential with the studies used for the final evaluation, naturally contaminated feed should be tested in dose–response studies in animals.

As hidden and bound fumonisins have been detected in corn and corn products, the Committee recommended that further studies be performed to elaborate more appropriate analytical methods to obtain additional occurrence data and information on the effects of processing.

As dietary exposure to fumonisins may occur together with exposure to other mycotoxins, such as aflatoxins, well-designed laboratory and epidemiological studies are needed to assess interactions.

For evaluation of the co-occurrence, in food and feed, of fumonisins with other mycotoxins, levels of fumonisins and other mycotoxins must be provided at the level of the individual analytical sample (i.e. not aggregate data).

Additional data on fumonisin distribution in corn food products should be collected in order to establish appropriate sampling procedures.

To validate the potential candidate urinary FB_1 level for a human biomarker of short-term exposure, large-scale human studies that indicate a well-characterized dose–response relationship between urinary FB_1 level and dietary fumonisin exposures are needed. A biomarker for long-term exposure is also needed.

To investigate the association of fumonisin exposure with oesophageal cancer risk, child growth impairment and NTDs in humans, studies on fumonisin

exposure and incidence of these conditions in individuals (such as a cohort or case–control study) are needed. These studies should use a validated fumonisin exposure biomarker and control for confounders and for known risk factors.

12. REFERENCES

Abbas HK, Shier WT, Cartwright RD (2004). Effect of heat stress on aflatoxin and fumonisin production in corn (maize, *Zea mays*) in Arkansas. *Phytopathology*, 94(Suppl. 6):S1.

Abbas HK, Shier WT, Cartwright RD (2007). Effect of temperature, rainfall and planting date on aflatoxin and fumonisin contamination in commercial Bt and non-Bt corn hybrids in Arkansas. *Phytoprotection*, 88:41–50.

Abbas HK et al. (2002). Aflatoxin and fumonisin contamination of commercial corn (*Zea mays*) hybrids in Mississippi. *Journal of Agricultural and Food Chemistry*, 50(18):5246–5254.

Abbas HK et al. (2006). Aflatoxin and fumonisin contamination of corn (maize, *Zea mays*) hybrids in Arkansas. *Crop Protection*, 25(1):1–9.

Abdel Nour AM et al. (2007). Folate receptor and human reduced folate carrier expression in HepG2 cell line exposed to fumonisin B_1 and folate deficiency. *Carcinogenesis*, 28:2291–2297.

Abdel-Wahhab MA et al. (2004). Prevention of fumonisin-induced maternal and developmental toxicity in rats by certain plant extracts. *Journal of Applied Toxicology*, 24:469–474.

Abnet CC et al. (2001). Sphingolipids as biomarkers of fumonisin exposure and risk of esophageal squamous cell carcinoma in China. *Cancer Causes and Control*, 12:821–828.

Afolabi CG et al. (2006). Effect of sorting on incidence and occurrence of fumonisins and *Fusarium verticillioides* on maize from Nigeria. *Journal of Food Protection*, 69(8):2019–2023.

Akiyama H et al. (1995). Liquid chromatographic determination of fumonisins B_1 and B_2 in corn by post column derivatization with 4-(*N,N*-dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole (DBD-F). *Journal of the Food Hygienic Society of Japan*, 36:77–81.

Al-Hazmi NA (2009). Occurrence of fumonisin B_1 in imported and local corn based snacks collected from Jeddah, Saudi Arabia. *Global Journal of Biotechnology & Biochemistry*, 4(2):193–200.

Aoyama K, Ishikuro E (2007). Fumonisin contamination of feeds in Japan. *Mycotoxins*, 57(1):11–16 (in Japanese).

Aoyama K et al. (2010). Four-year surveillance for ochratoxin A and fumonisins in retail foods in Japan. *Journal of Food Protection*, 73(2):344–352.

Ariño A, Estopañan G, González-Cabo JF (2007). Natural occurrence of *Fusarium* species, fumonisin production by toxigenic strains and concentrations of fumonisins B_1 and B_2 in conventional and organic maize grown in Spain. *Journal of Food Protection*, 70(1):151–156.

Ariño A et al. (2007). Estimation of dietary intakes of fumonisins B_1 and B_2 from conventional and organic corn. *Food Control*, 18:1058–1062.

Atukwase A, Kaaya AN, Muyanja C (2009). Factors associated with fumonisin contamination of maize in Uganda. *Journal of the Science of Food and Agriculture*, 89(14):2393–2398.

Avantaggiato G et al. (2003). Effects of Ruffini processing on fumonisins from ^{14}C-labeled toxins produced in cultured corn kernels. *Journal of Food Protection*, 66:1873–1878.

Aziz NH et al. (2007). Control of *Fusarium* moulds and fumonisin B_1 in seeds by gamma-irradiation. *Food Control*, 18(11):1337–1342.

Bakker MI et al. (2003). *Risk assessment of fumonisin B_1 in the Netherlands*. Bilthoven, the Netherlands, National Institute for Public Health and the Environment (RIVM) (Report No. 310301001).

Banczerowski P et al. (2008). Functional pathology of brain development caused by food contaminant fumonisin B-1. *Cereal Research Communications*, 36(Suppl. S, Part 3):1891–1894.

Banczerowski-Pelyhe I et al. (2008). Prenatal dietary fumonisin B-1 exposure may modify learning processes in rat offspring. *Cereal Research Communications*, 36(Suppl. S, Part 3):1947–1950.

Bankole SA, Mabekoje OO (2004). Occurrence of aflatoxins and fumonisins in preharvest maize from south-western Nigeria. *Food Additives and Contaminants*, 21(3):251–255.

Bankole SA, Mabekoje OO, Enikuomehin OA (2003). *Fusarium* spp. and fumonisin B_1 in stored maize from Ogun State, Nigeria. *Tropical Science*, 43:76–79.

Bartók T et al. (2006). Detection of new fumonisin mycotoxins and fumonisin-like compounds by reversed-phase high-performance liquid chromatography/electrospray ionization ion trap mass spectrometry. *Rapid Communications in Mass Spectrometry*, 20:2447–2462.

Bartók T et al. (2008). A new type of fumonisin series appeared on the scene of food and feed safety. *Cereal Research Communications*, 36:315–319.

Bartók T et al. (2010a). Identification of the first fumonisin mycotoxins with three acyl groups by ESI-ITMS and ESI-TOFMS following RP-HPLC separation: palmitoyl, linoleoyl and oleoyl EFB_1 fumonisin isomers from a solid culture of *Fusarium verticillioides*. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 27(12):1714–1723.

Bartók T et al. (2010b). Detection and characterization of twenty-eight isomers of fumonisin B_1 (FB_1) mycotoxin in a solid rice culture infected with *Fusarium verticillioides* by reversed-phase high-performance liquid chromatography/electrospray ionization time-of-flight and ion trap mass spectrometry. *Rapid Communications in Mass Spectrometry*, 24(1):35–42.

Batatinha MJM et al. (2007). Fumonisins in brewers grain (barley) used as dairy cattle feed in the State of Bahia, Brazil. *Food Control*, 18(5):608–612.

Beekrum S et al. (2003). Naturally occurring phenols: a detoxification strategy for fumonisin B_1. *Food Additives and Contaminants*, 20(5):490–493.

Beltrán E et al. (2009). Determination of mycotoxins in different food commodities by ultra-high-pressure liquid chromatography coupled to triple quadrupole mass spectrometry. *Rapid Communications in Mass Spectrometry*, 23:1801–1809.

Benedetti R et al. (2006). Degradation of fumonisin B_1 by a bacterial strain isolated from soil. *Biodegradation*, 17:31–38.

Berthiller F et al. (2009). Formation, determination and significance of masked and other conjugated mycotoxins. *Analytical and Bioanalytical Chemistry,* 395:1243–1252.

Bhandari N, Sharma RP (2002). Fumonisin B_1–induced alterations in cytokine expression and apoptosis signaling genes in mouse liver and kidney after an acute exposure. *Toxicology*, 172(2):81–92.

Binder EM et al. (2007). Worldwide occurrence of mycotoxins in commodities, feeds and feed ingredients. *Animal Feed Science and Technology*, 137:265–282.

Bird CB et al. (2002). Determination of total fumonisins in corn by competitive direct enzyme-linked immunosorbent assay: collaborative study. *Journal of AOAC International*, 85:404–410.

Bittencourt ABF et al. (2005). Mycotoxin occurrence in corn meal and flour trades in Sao Paulo, Brazil. *Food Control*, 16:117–120.

Black RE et al. (2008). Maternal and child undernutrition: global and regional exposures and health consequences. *Lancet*, 19:243–260.

Bondy GS et al. (2010). *Effects of long term exposure to fumonisin B_1 (FB_1) on wild type and p53+/– transgenic mice.* Ottawa, Ontario, Canada, Health Canada, Health Products and Food Branch, Food Directorate, Bureau of Chemical Safety, Toxicology Research Division (unpublished).

Boon PE et al. (2009). *Risk assessment of the dietary exposure to contaminants and pesticide residues in young children in the Netherlands.* Bilthoven, the Netherlands, National Institute for Public Health and the Environment (RIVM) (Report No. 350070002).

Bothast RJ et al. (1992). Fate of fumonisin B_1 in naturally contaminated corn during ethanol fermentation. *Applied and Environmental Microbiology*, 58:233–236.

Bouhet S et al. (2004). The mycotoxin fumonisin B_1 alters the proliferation and barrior function of porcine intestinal epithelial cells. *Journal of Toxicological Sciences*, 77:165–171.

Bouhet S et al. (2006). Mycotoxin fumonisin B_1 selectively down-regulates the basal IL-8 expression in pig intestine: in vivo and in vitro studies. *Food and Chemical Toxicology*, 44(10):1768–1773.

Brera C et al. (2004). Effect of industrial processing on distribution of fumonisin B_1 in dry milling corn fractions. *Journal of Food Protection*, 67(6):1261–1266.

Broggi LE et al. (2002). Distribution of fumonisins in dry-milled corn fractions in Argentina. *Food Additives and Contaminants*, 19(5):465–469.

Broggi L et al. (2007). Natural occurrence of aflatoxins, deoxynivalenol, fumonisins and zearalenone in maize from Entre Ríos Province, Argentina. *Mycotoxin Research*, 23(2):59–64.

Bruns HA (2003). Controlling aflatoxin and fumonisin in maize by crop management. *Toxin Reviews*, 22(2–3):153–173.

Bruns HA, Abbas HK (2005). Responses of short-season corn hybrids to a humid subtropical environment. *Agronomy Journal*, 97(2):446–451.

Bullerman LB, Bianchini A (2007). Stability of mycotoxins during food processing. *International Journal of Food Microbiology*, 119(1–2):140–146.

Bullerman LB et al. (2008). Reduction of fumonisin B_1 in corn grits by single-screw extrusion. *Journal of Agricultural and Food Chemistry*, 56(7):2400–2405.

Burger HM et al. (2007). Altered lipid parameters in hepatic subcellular membrane fractions induced by fumonisin B_1. *Lipids*, 42(3):249–261.

Burger HM et al. (2010). Dietary fumonisin exposure in a rural population of South Africa. *Food and Chemical Toxicology*, 48(8–9):2103–2108.

Burns TD et al. (2008). Fumonisin concentrations and in vivo toxicity of nixtamalized *Fusarium verticillioides* culture material: evidence for fumonisin–matrix interactions. *Food and Chemical Toxicology*, 46:2841–2848.

Busman M, Kendra D (2008). *Determination of fumonisin mycotoxins in ethanol by-products by liquid chromatography–mass spectrometry.* Presented at the 235th American Chemical Society National Meeting, New Orleans, LA, USA, 6–10 April 2008 (ACS National Meeting Book of Abstracts; http://oasys2.confex.com/acs/235nm/techprogram/P1152836.HTM).

Cai Q, Tang L, Wang JS (2007). Validation of fumonisin biomarkers in F344 rats. *Toxicology and Applied Pharmacology*, 225:28–39.

Caldas ED, Silva ACS (2007). Mycotoxins in corn-based food products consumed in Brazil: an exposure assessment for fumonisins. *Journal of Agricultural and Food Chemistry*, 55(19):7974–7980.

Cano-Sancho G et al. (2010). Biomonitoring of *Fusarium* spp. Mycotoxins: perspectives for an individual exposure assessment tool. *Food Science and Technology International*, 16:266–276.

Carlson DB et al. (2001). Fumonisin B_1 promotes aflatoxin B_1 and *N*-methyl-*N'*-nitro-nitrosoguanidine initiated liver tumors in rainbow trout. *Toxicology and Applied Pharmacology*, 172(1):29–36.

Casado MR et al. (2010). A short geostatistical study of the three-dimensional spatial structure of fumonisins in stored maize. *World Mycotoxin Journal*, 3(1):95–103.

CAST (2002). *Mycotoxins: risks in plant, animal and human systems.* Ames, IA, USA, Council for Agricultural Science and Technology (Task Force Report No. 139).

Castells M et al. (2005). Fate of mycotoxins in cereals during extrusion cooking: a review. *Food Additives and Contaminants*, *22*(2):150–157.

Castells M et al. (2008). Distribution of fumonisins and aflatoxins in corn fractions during industrial cornflake processing. *International Journal of Food Microbiology*, 123(1–2):81–88.

Castells M et al. (2009). Reduction of fumonisin B_1 in extruded corn breakfast cereals with salt, malt and sugar in their formulation. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 26(4):512–517.

Castelo MM et al. (2001). Loss of fumonisin B_1 in extruded and baked corn-based foods with sugars. *Journal of Food Science,* 66(3):416–421.

Cavaglieri LR et al. (2009). Fungi and natural incidence of selected mycotoxins in barley rootlets. *Journal of Stored Products Research*, 45(3):147–150.

Cavaliere C et al. (2005). Development of a multiresidue method for analysis of major *Fusarium* mycotoxins in corn meal using liquid chromatographic/tandem mass spectrometry. *Rapid Communications in Mass Spectrometry*, 19:2085–2093.

Cavaliere C et al. (2007). Mycotoxins produced by *Fusarium* genus in maize: determination by screening and confirmatory methods based on liquid chromatography tandem mass spectrometry. *Food Chemistry*, 105:700–710.

Chalfant CE, Spiegel S (2005). Sphingosine 1-phosphate and ceramide 1-phosphate: expanding roles in cell signaling. *Journal of Cell Science*, 118:4605–4612.

Chelule PK et al. (2001). Exposure of rural and urban populations in KwaZulu Natal, South Africa, to fumonisin B_1 in maize. *Environmental Health Perspectives*, 109(3):253–256.

Chelule PK et al. (2010). Lactic acid fermentation improves the quality of amahewu, a traditional South African maize-based porridge. *Food Chemistry*, 122(3):656–661.

Chulze SN (2010). Strategies to reduce mycotoxin levels in maize during storage: a review. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 27(5):651–657.

Chung SH et al. (2008). Fumonisin contamination in corn and processed corn products commercialized in Korea. *Cereal Research Communications*, 36:353–355.

Cirillo T et al. (2003a). Natural co-occurrence of deoxynivalenol and fumonisins B_1 and B_2 in Italian marketed foodstuffs. *Food Additives and Contaminants*, 20:566–571.

Cirillo T et al. (2003b). Evaluation of conventional and organic Italian foodstuffs for deoxynivalenol and fumonisins B_1 and B_2. *Journal of Agricultural and Food Chemistry*, 51(27):8128–8131.

Collins TF et al. (2006). Effects of aminopentol on in utero development in rats. *Food and Chemical Toxicology*, 44:161–169.

Constable PD et al. (2003). Fumonisin-induced blockade of ceramide synthase in sphingolipid biosynthetic pathway alters aortic input impedance spectrum of pigs. *American Journal of Physiology*, 284(6):H2034–H2044.

Constable PD et al. (2005). Serum sphingosine-1-phosphate and sphinganine-1-phosphate are elevated in horses exposed to fumonisin B_1. *AOAC International Midwest Section Final Program*, pp. 63–64.

Cortez-Rocha MO et al. (2002). Effect of extrusion processing on fumonisin B_1 and hydrolyzed fumonisin B_1 in contaminated alkali-cooked corn. *Bulletin of Environmental Contamination and Toxicology*, 69(4):471–478.

Cortez-Rocha MO et al. (2005). Occurrence of fumonisin B_1 and hydrolyzed fumonisin B_1 in Mexican nixtamalized cornmeal. *Bulletin of Environmental Contamination and Toxicology*, 74(1):73–77.

Creppy EE et al. (2004). Synergistic effects of fumonisin B_1 and ochratoxin A: are in vitro cytotoxicity data predictive of in vivo acute toxicity? *Toxicology*, 201:115–123.

Dall'Asta C et al. (2008). A LC/MS/MS method for the simultaneous quantification of free and masked fumonisins in corn and corn-based products. *World Mycotoxin Journal*, 1(3):1–10.

Dall'Asta C et al. (2009a). Free and bound fumonisins in gluten-free food products. *Molecular Nutrition & Food Research*, 53:492–499.

Dall'Asta C et al. (2009b). Difficulties in fumonisin determination: the issue of hidden fumonisins. *Analytical and Bioanalytical Chemistry*, 395(5):1335–1345.

Dall'Asta C et al. (2010). In vitro digestion assay for determination of hidden fumonisins in maize. *Journal of Agricultural and Food Chemistry*, 58:12 042–12 047.

Dambolena JS et al. (2010). Effects of menthol stereoisomers on the growth, sporulation and fumonisin B_1 production of *Fusarium verticillioides*. *Food Chemistry*, 123:165–170.

D'Arco G et al. (2008). Analysis of fumonisins B_1, B_2 and B_3 in corn-based baby food by pressurized liquid extraction and liquid chromatography/tandem mass spectrometry. *Journal of Chromatography A*, 1209(1–2):188–194.

D'Arco G et al. (2009). Survey of fumonisins B_1, B_2 and B_3 in conventional and organic retail corn products in Spain and Italy and estimated dietary exposure. *Food Additives & Contaminants. Part B, Surveillance Communications*, 2(2):146–153.

Da Silva M et al. (2008). Effect of the time interval from harvesting to the pre-drying step on natural fumonisin contamination in freshly harvested corn from the State of Paraná, Brazil. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 25(5):642–649.

De Angelis I et al. (2005). Absorption of fumonisin B_1 and aminopentol on an in vitro model of intestinal epithelium: the role of P-glycoprotein. *Toxicon*, 45:285–291.

de Castro MFPM et al. (2004). Fumonisins in Brazilian corn-based foods for infant consumption. *Food Additives and Contaminant*s, 21(7):693–699.

Decker L, ffrench-Constant C (2004). Lipid rafts and integrin activation regulate oligodendrocyte survival. *Journal of Neuroscience*, 24:3816–3825.

De Girolamo A, Solfrizzo M, Visconti A (2001). Effect of processing on fumonisin concentration in corn flakes. *Journal of Food Protection*, 64(5):701–705.

De Girolamo AM et al. (2001). Comparison of different extraction and clean-up procedures for the determination of fumonisins in maize and maize-based food products. *Food Additives and Contaminants*, 18:59–67.

De Girolamo A et al. (2010). Determination of fumonisins B_1 and B_2 in maize-based baby food products by HPLC with fluorimetric detection after immunoaffinity column clean-up. *World Mycotoxin Journal*, 3(2):135–146.

de la Campa R, Miller JD, Hendricks K (2004). Fumonisin in tortillas produced in small-scale facilities and effect of traditional masa production methods on this mycotoxin. *Journal of Agricultural and Food Chemistry*, 52(14):4432–4437.

de la Campa R et al. (2005). Modeling effects of environment, insect damage, and Bt genotypes on fumonisin accumulation in maize in Argentina and the Philippines. *Mycopathologia*, 159(4):539–552.

DeLorenzi L et al. (2005). Genotoxic activity of the fumonisin B_1 mycotoxin in cultures of bovine lymphocytes. *Italian Journal of Animal Science*, 4:395–402.

del Rio Garcia JC et al. (2007). [Evaluation of the cytotoxicity of aflatoxin and fumonisin in swine intestinal cells.] *Revista iberoamericana de micologia: organo de la Asociacion Espanola de Especialistas en Micologia*, 24(2):136–141 (in Spanish).

Deshmukh S et al. (2005). Individual and combined effects of *Fusarium moniliforme* culture material, containing known levels of fumonisin B_1, and *Salmonella gallinarum* infection on liver of Japanese quail. *Avian Diseases*, 49:592–600.

Deshmukh S et al. (2007). Pathologic changes in extrahepatic organs and agglutinin response to *Salmonella gallinarum* infection in Japanese quail fed *Fusarium verticillioides* culture material containing known levels of fumonisin B_1. *Avian Diseases*, 51:705–712.

De Smet D et al. (2009). Molecularly imprinted solid-phase extraction of fumonisin B analogues in bell pepper, rice and corn flakes. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 26(6):874–884.

Devriendt B et al. (2009). The food contaminant fumonisin B_1 reduces the maturation of porcine $CD11R1^+$ intestinal antigen presenting cells and antigen-specific immune responses, leading to a prolonged intestinal ETEC infection. *Veterinary Research*, 40(4):40.

Dilkin P et al. (2003). Toxicological effects of chronic low doses of aflatoxin B_1 and fumonisin B_1–containing *Fusarium moniliforme* culture material in weaned piglets. *Food Chemistry*, 41:1345–1353.

Dilkin P et al. (2010). Toxicokinetics and toxicological effects of a single oral dose of fumonisin B_1 containing *Fusarium verticillioides* culture material in weaned pigs. *Chemico-Biological Interactions*, 185:157–162.

Di Mavungu JD et al. (2009). LC-MS/MS multi-analyte method for mycotoxin determination in food supplements. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 26:885–895.

Direito GM et al. (2009). Evaluation of sphingolipids in Wistar rats treated to prolonged and single oral doses of fumonisin B_1. *International Journal of Molecular Science*, 10:50–61.

Domijan AM et al. (2006). Standard and Fpg-modified comet assay in kidney cells of ochratoxin A– and fumonisin B_1–treated rats. *Toxicology*, 222(1–2):53–59.

Domijan AM et al. (2007a). Fumonisin B_1: oxidative status and DNA damage in rats. *Toxicology*, 232(3):163–169.

Domijan AM et al. (2007b). The involvement of oxidative stress in ochratoxin A and fumonisin B_1 toxicity in rats. *Molecular Nutrition & Food Research*, 51(9):1147–1151.

Domijan AM et al. (2008). Early toxic effects of fumonisin B_1 in rat liver. *Human and Experimental Toxicology*, 27(12):895–900.

D'Ovidio KL et al. (2007). Effects of irradiation on fungi and fumonisin B_1 in corn, and of microwave-popping on fumonisins in popcorn. *Food Additives and Contaminants*, 24(7):735–743.

Dowd PF, Johnson ET (2010). Field incidence of mycotoxins in commercial popcorn and potential environmental influences. *Mycotoxin Research*, 26(1):15–22.

Duvick J (2001). Prospects for reducing fumonisin contamination of maize through genetic modification. *Environmental Health Perspectives*, 109:337–342.

Duvick J, Rood T, Wang X (1998). *Fumonisin detoxification enzymes*. United States Patent 5716820 (http://www.freepatentsonline.com/5716820.pdf).

Dvorak NJ et al. (2008). Fumonisin mycotoxin contamination of corn-based foods consumed by potentially pregnant women in southern California. *Journal of Reproductive Medicine*, 53(9):672–676.

Eaton DL et al. (2011). Hepatotoxic mycotoxins. In: Roth RA, Ganey PE, eds. *Comprehensive toxicology*, 2nd ed. *Vol. 9. Hepatic toxicology*. Amsterdam, the Netherlands, Elsevier, pp. 527–569.

EC (2006). Commission Regulation (EC) No. 401/2006 of 23 February 2006 laying down the sampling methods and the methods of analysis for the official control of the levels of *Fusarium* toxins in foodstuffs. *Official Journal of the European Union*, L70:12–34.

EFSA (2005). Opinion of the Scientific Panel on Contaminants in Food Chain on a request from the Commission related to fumonisins as undesirable substances in animal feed. *The EFSA [European Food Safety Authority] Journal*, 235:1–32.

Ehrlich V et al. (2002). Fumonisin B_1 is genotoxic in human derived hepatoma (HepG2) cells. *Mutagenesis*, 17(3):257–260.

Engelhardt G, Barthel J, Sparrer D (2006). *Fusarium* mycotoxins and ochratoxin A in cereals and cereal products. *Molecular Nutrition & Food Research*, 50:401–405.

Enongene EN et al. (2002). Persistence and reversibility of the elevation in free sphingoid bases induced by fumonisin inhibition of ceramide synthase. *Toxicological Sciences*, 67:173–181.

Ewuola EO (2009). Organ traits and histopathology of rabbits fed varied levels of dietary fumonisin B_1. *Journal of Animal Physiology and Animal Nutrition*, 93(6):726–731.

Ewuola EO, Egbunike GN (2008). Haematological and serum biochemical response of growing rabbit bucks fed dietary fumonisin B_1. *African Journal of Biotechnology*, 7(23):4304–4309.

Ewuola EO, Egbunike GN (2010a). Effects of dietary fumonisin B_1 on the onset of puberty, semen quality, fertility rates and testicular morphology in male rabbits. *Reproduction*, 139(2):439–445.

Ewuola EO, Egbunike GN (2010b). Gonadal and extra-gonadal sperm reserves and sperm production of pubertal rabbits fed dietary fumonisin B_1. *Animal Reproduction Science*, 119(3–4):282–286.

Ewuola EO et al. (2008). Physiological response of rabbit bucks to dietary fumonisin: performance, haematology and serum biochemistry. *Mycopathologia*, 165(2):99–104.

Faberi A et al. (2005). Determination of type B fumonisin mycotoxins in maize and maize-based products by liquid chromatography/tandem mass spectrometry using a QqQlinear ion trap mass spectrometer. *Rapid Communications in Mass Spectrometry*, 19:275–282.

Fandohan P et al. (2005). Fate of aflatoxins and fumonisins during the processing of maize into food products in Benin. *International Journal of Food Microbiology*, 98(3):249–259.

FAO/IAEA (2001). *FAO/IAEA manual on the application of HACCP system in mycotoxin prevention and control*. Rome, Italy, Food and Agriculture Organization of the United Nations and International Agency for Atomic Energy, FAO/IAEA Training and Reference Centre for Food and Pesticide Control, pp. 1–130 (FAO Food and Nutrition Paper No. 73; http://www.fao.org/docrep/005/y1390e/y1390e00.htm).

FAO/WHO (2010a). *Proposed draft maximum levels for fumonisins in maize and maize products and associated sampling plans*. Rome, Italy, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, Codex Committee on Contaminants in Foods (CX/CF 10/4/8; ftp://ftp.fao.org/codex/cccf4/cf04_08_Add1e.pdf).

FAO/WHO (2010b). *Report of the Fourth Session of the Codex Committee on Contaminants in Foods, Izmir, Turkey, 26–30 April 2010.* Rome, Italy, Food and Agriculture Organization of the United Nations and World Health Organization, Joint FAO/WHO Food Standards Programme, Codex Alimentarius Commission, 2010 (ALINORM 10/33/41; http://www.codexalimentarius.net/web/archives.jsp?year=10).

Federico FJ et al. (2010). Natural occurrence of fumonisins in corn puff in Argentina. *Mycotoxin Research*, 26:273–278.

Fernandez-Surumay G et al. (2005). Fumonisin B–glucose reaction products are less toxic when fed to swine. *Journal of Agricultural and Food Chemistry*, 53(10):4264–4271.

Fink-Gremmels J (2008). Mycotoxins in cattle feeds and carry-over to dairy milk: a review. *Food Additives and Contaminants*, 25(2):172–180.

Fodor J et al. (2007). In vitro microbial metabolism of fumonisin B_1. *Food Additives and Contaminants*, 24:416–420.

Fodor J et al. (2008). Absorption, distribution and elimination of fumonisin B_1 metabolites in weaned piglets. *Food Additives and Contaminants*, 25(1):88–96.

Foreman JH et al. (2004). Neurological abnormalities and cerebrospinal fluid changes in horses administered fumonisin B_1 intravenously. *Journal of Veterinary Internal Medicine*, 18:223–230.

Frenich AG et al. (2009). Simple and high-throughput method for the multimycotoxin analysis in cereals and related foods by ultra-high performance liquid chromatography/tandem mass spectrometry. *Food Chemistry*, 117:705–712.

Frisvad J et al. (2006). Important mycotoxins and the fungi which produce them. *Advances in Experimental Medicine and Biology*, 571:3–31.

Frisvad JC et al. (2007). Fumonisin B_2 production by *Aspergillus niger*. *Journal of Agricultural and Food Chemistry*, 55(23):9727–9732.

Gazzotti T et al. (2009). Determination of fumonisin B_1 in bovine milk by LC-MS/MS. *Food Control*, 20:1171–1174.

Gazzotti T et al. (2011). Analysis of fumonisins B_1, B_2 and their hydrolysed metabolites in pig liver by LC-MS/MS. *Food Chemistry*, 125:1379–1384.

Gbore FA (2009a). Growth performance and puberty attainment in growing pigs fed dietary fumonisin B. *Journal of Animal Physiology and Animal Nutrition*, 93:761–767.

Gbore FA (2009b). Reproductive organ weights and semen quality of pubertal boars fed dietary fumonisin B_1. *Animal*, 3(8):1133–1137.

Gbore FA (2010). Brain and hypophyseal acetylcholinesterase activity of pubertal boars fed dietary fumonisin B_1. *Journal of Animal Physiology and Animal Nutrition*, 94(5):123–129.

Gbore FA, Akele O (2010). Growth performance, haematology and serum biochemistry of female rabbits (*Oryctolagus cuniculus*) fed dietary fumonisin. *Veterinary Archives*, 80(3):431–443.

Gbore FA, Egbunike GN (2007). Influence of dietary fumonisin B_1 on nutrient utilization by growing pigs. *Livestock Research for Rural Development*, 19(7), Article #93 (http://www.lrrd.org/lrrd19/7/gbor19093.htm).

Gbore FA, Egbunike GN (2008). Testicular and epididymal sperm reserves and sperm production of pubertal boars fed dietary fumonisin B_1. *Animal Reproduction Science*, 105(3–4):392–397.

Gbore FA, Yinusa RI, Salleh B (2010). Evaluation of subchronic dietary fumonisin B_1 on nutrient digestibility and growth performance of rats. *African Journal of Biotechnology*, 9(38):6442–6447.

Gelderblom WCA, Marasas WFO (2011). Controversies in fumonisin mycotoxicology and risk assessment. *Human and Experimental Toxicology* [Epub ahead of print].

Gelderblom WCA et al. (1991). Toxicity and carcinogenicity of the *Fusarium moniliforme* metabolite, fumonisin B_1, in rats. *Carcinogenesis*, 12:1247–1251.

Gelderblom WCA et al. (2001). Cancer initiation by fumonisin B_1 in rat liver—Role of cell proliferation. *Cancer Letters*, 169(2):127–137.

Gelderblom WCA et al. (2002a). Interaction of fumonisin B_1 and aflatoxin B_1 in a short-term carcinogenesis model in rat liver. *Toxicology*, 171(2–3):161–173.

Gelderblom WCA et al. (2002b). Lipids and Δ6-desaturase activity alterations in rat liver microsomal membranes induced by fumonisin B_1. *Lipids*, 37(9):869–877.

Gelderblom WCA et al. (2004). Fumonisin contamination of a corn sample associated with induction of hepatocarcinogenesis in rats—Role of dietary deficiencies. *Food and Chemical Toxicology*, 42:471–479.

Gelderblom WCA et al. (2007). Structure and natural occurrence of stereoisomers of the fumonisin B series mycotoxins. *Journal of Agricultural and Food Chemistry*, 55:4388–4394.

Gelderblom WCA et al. (2008). Cancer initiating properties of fumonisin B_1 in a short-term rat liver carcinogenesis assay. *Toxicology*, 250(2–3):89–95.

Gelineau-Van Waes J et al. (2005). Maternal fumonisin exposure and risk for neural tube defects: disruption of sphingolipid metabolism and folate transport in an in vivo mouse model. *Birth Defects Research*, 73:487–497.

Gelineau-Van Waes J et al. (2009). Maternal fumonisin exposure as a risk factor for neural tube defects. *Advances in Food Nutrition Research*, 56:145–181.

Ghiasian SA et al. (2006). Incidence of *Fusarium verticillioides* and levels of fumonisins in corn from main production areas in Iran. *Journal of Agricultural and Food Chemistry*, 54(16):6118–6122.

Ghiasian SA et al. (2009). Occurrence of fumonisins in maize imported into Iran during 2001–2002. *Mycotoxin Research*, 25(1):25–28.

Goertz A et al. (2010). *Fusarium* species and mycotoxin profiles on commercial maize hybrids in Germany. *European Journal of Plant Pathology*, 128(1):101–111.

Gong H-Z et al. (2009). Occurrence of fumonisin B_1 in corn from the main corn-producing areas of China. *Mycopathologia*, 167(1):31–36.

Gong YY et al. (2002). Dietary aflatoxin exposure and impaired growth in young children from Benin and Togo: cross sectional study. *British Medical Journal*, 325:20–21.

Gong YY et al. (2004). Postweaning exposure to aflatoxin results in impaired child growth: a longitudinal study in Benin, West Africa. *Environmental Health Perspectives*, 112:1334–1338.

Gong YY et al. (2008). Association between tortilla consumption and human urinary fumonisin B_1 levels in a Mexican population. *Cancer Epidemiology, Biomarkers & Prevention*, 17(3):688–694.

Goryacheva IY et al. (2007). Immunochemical methods for rapid mycotoxin detection: evolution from single to multiple analyte screening: a review. *Food Additives and Contaminants*, 24(10):1169–1183.

Grenier B et al. (2011). Individual and combined effects of subclinical doses of deoxynivalenol and fumonisins in piglets. *Molecular Nutrition & Food Research*, 55(5):761–771.

Griessler K et al. (2010). Occurrence of mycotoxins in southern Europe. *World Mycotoxin Journal*, 3(3):301–309.

Groopman JD, Kensler TW, Wild CP (2008). Protective interventions to prevent aflatoxin-induced carcinogenesis in developing countries. *Annual Review of Public Health*, 29:187–203.

Gwiazdowska D et al. (2008). Effects of *Propionibacterium* on the growth and mycotoxin production by some species of *Fusarium* and *Alternaria*. *Polish Journal of Microbiology*, 57:205–212.

Hall AJ, Wild CP (1994). Epidemiology of aflatoxin-related disease. In: Eaton DA, Groopman JD, eds. *The toxicology of aflatoxins: human health, veterinary and agricultural significance.* San Diego, CA, USA, Academic Press, pp. 233–258.

Halloy DJ et al. (2005). Oral exposure to culture material extract containing fumonisins predisposes swine to the development of pneumonitis caused by *Pasteurella multocida*. *Toxicology*, 213:34–44.

Hammond BG et al. (2004). Lower fumonisin mycotoxin levels in the grain of Bt corn grown in the United States in 2000–2002. *Journal of Agricultural and Food Chemistry*, 52(5):1390–1397.

Hard GC, Seely JC (2005). Recommendations for the interpretation of renal tubule proliferative lesions occurring in rat kidneys with advanced chronic progressive nephropathy (CPN). *Toxicologic Pathology*, 33(6):641–649.

Harvey RB et al. (2002). Toxicity of fumonisin from *Fusarium verticillioides* culture material and moniliformin from *Fusarium fujikuroi* culture material when fed singly and in combination to growing barrows. *Journal of Food Protection*, 65(2):373–377.

Haschek WM, Voss KA, Beasley VR (2002). Selected mycotoxins affecting animal and human health. In: Haschek W, Rousseaux C, Wallig M, eds. *Handbook of toxicologic pathology*, 2nd ed. *Vol. 1*. Academic Press, pp. 645–699.

Hassan AM et al. (2010). *Aquilegia vulgaris* L. extract counteracts oxidative stress and cytotoxicity of fumonisin in rats. *Toxicon*, 56(1):8–18.

Haubruge E et al. (2003). Mycotoxins in stored barley (*Hordeum vulgare*) in Tibet Autonomous Region (People's Republic of China). *Mountain Research and Development*, 23(3):284–287.

He Q, Riley RT, Sharma RP (2005). Myriocin prevents fumonisin B_1–induced sphingoid base accumulation in mice liver without ameliorating hepatotoxicity. *Food and Chemical Toxicology*, 43(6):969–979.

Heinl S et al. (2010). Degradation of fumonisin B_1 by the consecutive action of two bacterial enzymes. *Journal of Biotechnology*, 145:120–129.

Henry MH, Wyatt RD (2001). The toxicity of fumonisin B_1, B_2, and B_3, individually and in combination, in chicken embryos. *Poultry Science*, 80(4):401–407.

Howard PC et al. (2002). Comparison of the toxicity of several fumonisin derivatives in a 28-day feeding study with female B6C3F1 mice. *Toxicology and Applied Pharmacology*, 185:153–165.

Hsaio SH et al. (2005). Effects of exogenous sphinganine, sphingosine, and sphingosine-1-phosphate on relaxation and contraction of porcine thoracic aortic and pulmonary arterial rings. *Toxicological Sciences*, 86:194–199.

Huls R, Zuiderent R, Ghosh D (2007). Analysis of mycotoxins in various cattle forages and food matrices with the TSQ quantum discovery MAX. *Mass Spectrometry*, Suppl:30.

Humpf HU, Voss KA (2004). Effects of thermal food processing on the chemical structures and toxicity of fumonisin mycotoxins. *Molecular Nutrition & Food Research*, 48:255–269.

IARC (2002) Fumonisin B_1. *IARC [International Agency for Research on Cancer] Monographs on the Evaluation of Carcinogenic Risk to Humans*, 82:275–366.

IARC (2008). *GLOBOCAN database*. Lyon, France, International Agency for Research on Cancer (http://globocan.iarc.fr, accessed April 2011).

Imgrund S et al. (2009). Adult ceramide synthase 2 (CERS2)-deficient mice exhibit myelin sheath defects, cerebellar degeneration, and hepatocarcinomas. *Journal of Biological Chemistry*, 284:33 549–33 560.

IPCS (2000). *Fumonisin B_1*. Geneva, Switzerland, World Health Organization, International Programme on Chemical Safety (Environmental Health Criteria 219; http://www.who.int/ipcs/publications/ehc/ehc_219_rev_1.pdf).

Jackson L, Jablonski J (2004). Fumonisins. In: Magan N, Olsen M, eds. *Mycotoxins in food: detection and control*. Cambridge, England, Woodhead Publishing Ltd, pp. 367–405.

Jackson LS et al. (2011). Reduction of fumonisin B_1 in corn grits by twin-screw extrusion. *Journal of Food Science*, 76(6):T150–T155.

Johansson AS et al. (2006). Predicting aflatoxin and fumonisin in shelled corn lots using poor-quality grade components. *Journal of the Association of Official Analytical Chemists*, 89:433–440.

Johnson VJ, Sharma RP (2001). Gender-dependent immunosuppression following subacute exposure to fumonisin B_1. *International Immunopharmacology*, 1(11):2023–2034.

Johnson VJ et al. (2001). Fumonisin B_1–induced immunosuppression in BALB/c mice is more pronounced in females than in males. *FASEB Journal*, 15(4):A566.

Jouany JP (2007). Methods for preventing, decontaminating and minimizing the toxicity of mycotoxins in feeds. *Animal Feed Science and Technology*, 137:342–362.

Kabak B (2008). The fate of mycotoxins during thermal food processing. *Journal of the Science of Food and Agriculture*, 89:549–554.

Kabak B, Dobson ADW (2009). Biological strategies to counteract the effects of mycotoxins. *Journal of Food Protection*, 72(9):2006–2016.

Karbancioglu-Güler F, Heperkan D (2009). Natural occurrence of fumonisin B_1 in dried figs as an unexpected hazard. *Food and Chemical Toxicology*, 47(2):289–292.

Kawashima LM, Valente Soares LM (2006). [Occurrence of fumonisin B_1, aflatoxins B_1, B_2, G_1 and G_2, ochratoxin A and zearalenone in corn products.] *Ciência e Tecnologia de Alimentos*, 26(3):516–521 (in Portuguese).

Kawashima LM, Vieira AP, Valente Soares LM (2007). Fumonisin B_1 and ochratoxin A in beers made in Brazil. *Ciência e Tecnologia de Alimentos*, 27(2):317–323.

Khayoon WS et al. (2010). A reversed phase high performance liquid chromatography method for the determination of fumonisins B_1 and B_2 in food and feed using monolithic column and positive confirmation by liquid chromatography/tandem mass spectrometry. *Analytica Chimica Acta*, 679(1–2):91–97.

Kim DH et al. (2006). Elevation of sphinganine 1-phosphate as a predictive biomarker for fumonisin exposure and toxicity in mice. *Journal of Toxicology and Environmental Health, Part A*, 69:2071–2082.

Kim DH et al. (2007). Elevation of sphingoid base 1-phosphate as a potential contributor to hepatotoxicity in fumonisin B_1–exposed mice. *Archives of Pharmaceutical Research*, 30:962–969.

Kim E-K, Scott PM, Lau BP-Y (2003). Hidden fumonisins in corn flake. *Food Additives and Contaminants*, 20:161–169.

Kim EK et al. (2002). Survey for fumonisin B_1 in Korean corn-based food products. *Food Additives and Contaminants*, 19(5):459–464.

Kimanya ME et al. (2008). Co-occurrence of fumonisins with aflatoxins in home-stored maize for human consumption in rural villages of Tanzania. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 25(11):1353–1364.

Kimanya ME et al. (2009). Exposure of infants to fumonisins in maize-based complementary foods in rural Tanzania. *Molecular Nutrition & Food Research*, 53(5):667–674.

Kimanya ME et al. (2010). Fumonisin exposure through maize in complementary foods is inversely associated with linear growth of infants in Tanzania. *Molecular Nutrition & Food Research*, 54(11):1659–1667.

Knudsen PB et al. (2011). Occurrence of fumonisins B_2 and B_4 in retail raisins. *Journal of Agricultural and Food Chemistry*, 59(2):772–776.

Kouadio JH et al. (2007). Effects of combinations of *Fusarium* mycotoxins on the inhibition of macromolecular synthesis, malondialdehyde levels, DNA methylation and fragmentation, and viability in Caco-2 cells. *Toxicon*, 49(3):306–317.

Kpodo KA (2001). *Fusaria and fumonisins in maize and fermented maize products from Ghana* [PhD Thesis]. Accra, Ghana, University of Ghana.

Kpodo K, Sorensen AK, Jakobsen M (2000). Fusaria and fumonisins in maize from Ghana and their co-occurrence with aflatoxins. *International Journal of Food Microbiology*, 61:147–157.

Kpodo KA et al. (2006). Exposure to fumonisins through kenkey—a Ghanaian fermented maize product. In: Njapau H et al., eds. *Mycotoxins and phycotoxins: advances in determination, toxicology and exposure management.* Wageningen, the Netherlands, Academic Publishers, pp. 209–216.

Krska R, Molinelli A (2009). Rapid test strips for analysis of mycotoxins in food and feed. *Analytical and Bioanalytical Chemistry*, 393:67–71.

Krska R et al. (2008). Mycotoxin analysis: an update. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 25(2):152–163.

Kushiro M et al. (2007). Liquid chromatographic determination of fumonisins B_1, B_2, and B_3 in rice. *Mycotoxins*, 57:95–104.

Kushiro M et al. (2009). Limited surveillance of fumonisins in brown rice and wheat harvested in Japan. *Journal of Food Protection*, 72(6):1327–1331.

Lambert R et al. (2001). A study of the minimum inhibitory concentration and mode of action of oregano essential oil, thymol and carvacrol. *Journal of Applied Microbiology*, 91:453–462.

Lattanzio VMT et al. (2007). Simultaneous determination of aflatoxins, ochratoxin A and *Fusarium* toxins in maize by liquid chromatography/tandem mass spectrometry after multitoxin immunoaffinity cleanup. *Rapid Communications in Mass Spectrometry*, 21:3253–3261.

Leblanc J-C et al. (2005). Estimated dietary exposure to principal food mycotoxins from the first French Total Diet Study. *Food Additives and Contaminants*, 22(7):652–672.

Ledoux DR et al. (2003). Individual and combined effects of the *Fusarium* mycotoxins fumonisin B_1 and moniliformin in broiler chicks. *Avian Diseases*, 47(4):1368–1375.

Lefyedi ML, Taylor JRN (2006). Effect of dilute alkaline steeping on the microbial contamination, toxicity and diastatic power of sorghum malt. *Journal of the Institute of Brewing*, 112(2):108–116.

Lemmer ER et al. (2004). Fumonisin B_1–induced hepatocellular and cholangiocellular tumors in male Fischer 344 rats: potentiating effects of 2-acetylaminofluorene on oval cell proliferation and neoplastic development in a discontinued feeding study. *Carcinogenesis*, 25(7):1257–1264.

Lerda D et al. (2005). Fumonisins in foods from Cordoba (Argentina), presence and genotoxicity. *Food and Chemical Toxicology*, 43(5):691–698.

Lessard M et al. (2009). Intestinal physiology and peptidase activity in male pigs are modulated by consumption of corn culture extracts containing fumonisins. *Journal of Nutrition*, 139:1303–1307.

Li W, Herrman TJ, Dai SY (2010). Rapid determination of fumonisins in corn-based products by liquid chromatography/tandem mass spectrometry. *Journal of AOAC International*, 93(5):1472–1481.

Liesener K et al. (2010). Mycotoxins in horse feed. *Mycotoxin Research*, 26:23–30.

Ligler FS et al. (2003). Array biosensor for detection of toxins. *Analytical and Bioanalytical Chemistry*, 377:469–477.

Lino CM et al. (2006). Determination of fumonisins B_1 and B_2 in Portuguese maize and maize-based samples by HPLC with fluorescence detection. *Analytical and Bioanalytical Chemistry*, 384:1214–1220.

Lino CM et al. (2007). Occurrence of fumonisins B_1 and B_2 in broa, typical Portuguese maize bread. *International Journal of Food Microbiology*, 118(1):79–82.

Liu C et al. (2005). Occurrence of fumonisins B_1 and B_2 in asparagus from Shandong province, P.R. China. *Food Additives and Contaminants*, 22(7):673–676.

Liu F-M et al. (2005). Determination of fumonisins B_1 and B_2 in corn products. *Journal of Food and Drug Analysis*, 13(3):273–278.

Logrieco A et al. (2010). Natural occurrence of fumonisin B_2 in red wine from Italy. *Food Additives and Contaminants*, 27(8):1136–1141.

Loiseau N et al. (2007). Fumonisin B_1 exposure and its selective effect on porcine jejunal segment: sphingolipids, glycolipids and trans-epithelial passage disturbance. *Biochemical Pharmacology*, 74:144–152.

Lombaert GA et al. (2003). Mycotoxins in infant cereal foods from the Canadian retail market. *Food Additives and Contaminants*, 20(5):494–504.

López A et al. (2004). Aromatic plants essential oils activity on *Fusarium verticillioides* fumonisin B_1 production in corn grain. *Mycopathologia*, 158:343–349.

Luongo D et al. (2006). Interactive effects of fumonisin B_1 and α-zearalenol on proliferation and cytokine expression in Jurkat T cells. *Toxicology In Vitro*, 20(8):1403–1410.

Luongo D et al. (2008). Effects of four *Fusarium* toxins (fumonisin B_1, α-zearalenol, nivalenol and deoxynivalenol) on porcine whole-blood cellular proliferation. *Toxicon*, 52(1):156–162.

Maceyka M, Milstien S, Spiegel S (2009). Sphingosine-1-phosphate: the Swiss army knife of sphingolipid signaling. *Journal of Lipid Research*, 50:S272–S276.

Magro SL et al. (2011). Assessment of fumonisins B_1 and B_2 levels in commercial maize-based food products by liquid chromatography with fluorimetric detection and postcolumn chemical derivatization. *Journal of Food Science*, 76(1):T1–T4.

Makun HA et al. (2011). Natural multi-occurrence of mycotoxins in rice from Niger State, Nigeria. *Mycotoxin Research*, 27(2):97–104.

Manjula KK et al. (2009). Aflatoxin and fumonisin contamination of cassava products and maize grain from markets in Tanzania and Republic of the Congo. *Toxin Reviews*, 28:63–69.

Manova R, Mladenova R (2009). Incidence of zearalenone and fumonisins in Bulgarian cereal production. *Food Control*, 20:362–365.

Mansson M et al. (2010). Isolation and NMR characterization of fumonisin B_2 and a new fumonisin B_6 from *Aspergillus niger*. *Journal of Agricultural and Food Chemistry*, 58(2):949–953.

Maragos CM et al. (2001). Fluorescence polarization as a means for determination of fumonisins in maize. *Journal of Agricultural and Food Chemistry*, 49:596–602.

Marasas WFO et al. (2004). Fumonisins disrupt sphingolipid metabolism, folate transport, and neural tube development in embryo culture and in vivo: a potential risk factor for human neural tube defects among populations consuming fumonisin-contaminated maize. *Journal of Nutrition*, 134:711–716.

Marin DE et al. (2006). Sex-related differences in the immune response of weanling piglets exposed to low doses of fumonisin extract. *British Journal of Nutrition*, 95:1185–1192.

Marin DE et al. (2007). Fumonisin B_1 alters cell cycle progression and interleukin-2 synthesis in swine peripheral blood mononuclear cells. *Molecular Nutrition & Food Research*, 51(11):1406–1412.

Marin S et al. (2004). Fumonisin producing strains of *Fusarium*: a review of their ecophysiology. *Journal of Food Protection*, 61:1792–1805.

Martins HM, Guerra MM, Bernardo F (2006). Zearalenone, deoxynivalenol and fumonisins in mixed-feed for laying hens. *Mycotoxin Research*, 22(4):206–210.

Martins HM et al. (2008). Fumonisins and deoxynivalenol in corn-based food products in Portugal. *Food and Chemical Toxicology*, 46:2585–2587.

Marvasi L et al. (2006). Interaction between fumonisin B_1 and pig liver cytochromes P450. In: Njapau H et al., eds. *Mycotoxins and phycotoxins: advances in determination, toxicology and exposure management.* Wageningen, the Netherlands, Academic Publishers, pp. 135–143.

McKean C et al. (2006). Comparative acute and combinative toxicity of aflatoxin B_1 and fumonisin B_1 in animals and human cells. *Food and Chemical Toxicology*, 44(6):868–876.

Meca G et al. (2010). Formation of fumonisin B_1–glucose reaction product, in vitro cytotoxicity, and lipid peroxidation on kidney cells. *Journal of Agricultural and Food Chemistry*, 58(2):1359–1365.

Menniti AM, Gregori R, Neri F (2010). Activity of natural compounds on *Fusarium verticillioides* and fumonisins production in stored maize kernels. *International Journal of Food Microbiology*, 136:304–309.

Minervini F, Fornelli F, Flynn KM (2004). Toxicity and apoptosis induced by the mycotoxins nivalenol, deoxynivalenol and fumonisin B_1 in a human erythroleukemia cell line. *Toxicology In Vitro*, 18(1):21–28.

Miraglia M et al. (2005). The role of sampling in mycotoxin contamination: an holistic view. *Food Additives and Contaminants*, 22(Suppl. 1):31–36.

Missmer SA et al. (2006). Exposure to fumonisins and the occurrence of neural tube defects along the Texas–Mexico border. *Environmental Health Perspectives*, 114(2):237–241.

Mngadi PT, Govinden R, Odhav B (2008). Co-occurring mycotoxins in animal feeds. *African Journal of Biotechnology*, 7(13):2239–2243.

Mobio TA et al. (2003). Comparative study of the toxic effects of fumonisin B_1 in rat C6 glioma cells and p53-null mouse embryo fibroblasts. *Toxicology*, 183(1–3):65–75.

Mogensen JM et al. (2010). Production of fumonisin B_2 and B_4 by *Aspergillus niger* on grapes and raisins. *Journal of Agricultural and Food Chemistry*, 58:954–958.

Mokoena MP, Chelule PK, Gqaleni N (2005). Reduction of fumonisin B_1 and zearalenone by lactic acid bacteria in fermented maize meal. *Journal of Food Protection*, 68(10):2095–2099.

Molinelli A, Grossalber K, Krska R (2009). A rapid lateral flow test for the determination of total type B fumonisins in maize. *Analytical and Bioanalytical Chemistry*, 395:1309–1316.

Molinié A et al. (2005). Analysis of some breakfast cereals on the French market for their contents of ochratoxin A, citrinin and fumonisin B_1: development of a method for simultaneous extraction of ochratoxin A and citrinin. *Food Chemistry*, 92(3):391–400.

Monbaliu S et al. (2010). Occurrence of mycotoxins in feeds as analyzed by a multi-mycotoxin LC-MS/MS method. *Journal of Agricultural and Food Chemistry*, 58:66–71.

Moreno EC et al. (2009). Co-occurrence of mycotoxins in corn samples from the northern region of Paraná State, Brazil. *Food Chemistry*, 116(1):220–226.

Moreno Ramos C et al. (2010). Experimental trial of the effect of fumonisin B_1 and the PRRS virus in swine. *Journal of Animal and Veterinary Advances*, 9(9):1301–1310.

Motta EL, Scott PM (2009). Bioaccessibility of total bound fumonisin from corn flakes. *Mycotoxin Research*, 25:229–232.

Munkvold GP (2003). Mycotoxins in corn—occurrence, impact, and management. In: White PJ, Johnson LA, eds. *Corn: chemistry and technology*, 2nd ed. St. Paul, MN, USA, AAC, Inc., pp. 811–881.

Munkvold GP, Hellmich RL, Showers WB (1999). Comparison of fumonisin concentrations in kernels of transgenic Bt corn hybrids and nontransgenic hybrids. *Plant Disease*, 83:130–138.

Murphy PA et al. (1996). Effect of processing on fumonisin content of corn. In: Jackson LS, DeVries JW, Bullerman LB, eds. *Fumonisins in food.* New York, NY, USA, Plenum Press, pp. 323–334.

Muscarella M et al. (2008). Determination of a new analytical method for the determination of fumonisins B_1 and B_2 in food products based on high performance liquid chromatography and fluorimetric detection with post-column derivatization. *Journal of Chromatography A*, 1203:88–93.

Mwanza M et al. (2009). The cytotoxic effect of fumonisin B_1 and ochratoxin A on human and pig lymphocytes using the methyl thiazol tetrazolium (MTT) assay. *Mycotoxin Research*, 25(4):233–238.

Ndube N, Van der Westhuizen L, Shephard GS (2009). Determination of fumonisins in maize by HPLC with ultraviolet detection of *o*-phthaldialdehyde derivatives. *Mycotoxin Research*, 25(4):225–228.

Niderkorn V, Boudra H, Morgavi DP (2006). Binding of *Fusarium* mycotoxins by fermentative bacteria in vitro. *Journal of Applied Microbiology*, 101(4):849–856.

Niderkorn V et al. (2007). Screening of fermentative bacteria for their ability to bind and biotransform deoxynivalenol, zearalenone and fumonisins in an in vitro simulated corn silage model. *Food Additives and Contaminants*, 24(4):406–415.

Nielsen KF et al. (2009). Review of secondary metabolites and mycotoxins from the *Aspergillus niger* group. *Analytical and Bioanalytical Chemistry*, 395:1225–1242.

Nikièma PA et al. (2008). Fumonisin exposure and the sphinganine/sphingosine ratio in urine, serum and buccal cells in adults from Burkina Faso, West Africa. *World Mycotoxin Journal*, 1(4):483–491.

Nikièma PN et al. (2004). Fumonisin contamination of maize in Burkina Faso, West Africa. *Food Additives and Contaminants*, 21(9):865–870.

Njobeh PB et al. (2010). Simultaneous occurrence of mycotoxins in human food commodities in Cameroon. *Mycotoxin Research*, 26:47–57.

Noonim P et al. (2009). Fumonisin B_2 production by *Aspergillus niger* in Thai coffee beans. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 26(1):94–100.

NTP (2001). *NTP technical report on the toxicology and carcinogenesis studies of fumonisin B_1 (CAS No. 116355-83-0) in F344/N rats and B6C3F1 mice (feed studies).* Research Triangle Park, NC, USA, Department of Health and Human Services, Public Health Service, National Institutes of Health, National Toxicology Program (NTP Technical Report 496; NIH Publication No. 01-3955; http://ntp.niehs.nih.gov/ntp/htdocs/lt_rpts/tr496.pdf).

Nuryono A et al. (2002). A survey of fumonisins (B_1, B_2, B_3) in Indonesian corn-based food and feed samples. *Mycotoxin Research*, 18:117–120.

Odhav B et al. (2008). Modulating effects of fumonisin B_1 and ochratoxin A on leukocytes and messenger cytokines of the human immune system. *International Immunopharmacology*, 8(6):799–809.

Ofitserova M et al. (2009). Multiresidue mycotoxin analysis in corn grain by column high-performance liquid chromatography with postcolumn photochemical and chemical derivatization: single-laboratory validation. *Journal of AOAC International*, 92:15–25.

Ogido R et al. (2004). Effects of prolonged administration of aflatoxin B_1 and fumonisin B_1 in laying Japanese quail. *Poultry Science*, 83(12):1953–1958.

Oh KS, Scott PM, Chung S-H (2009). Incomplete recoveries of fumonisins present in naturally contaminated corn foods from an immunoaffinity column. *Journal of AOAC International*, 92:469–501.

Oliveira G et al. (2006). Mycobiota in poultry feeds and natural occurrence of aflatoxins, fumonisins and zearalenone in the Rio de Janeiro State, Brazil. *Mycopathologia*, 162(5):355–362.

Omurtag GZ (2001). Determination of fumonisin B_1 and B_2 in corn and corn-based products in Turkey by high-performance liquid chromatography. *Journal of Food Protection*, 64:1072–1075.

Omurtag GZ, Yazicioğlu D (2004). Determination of fumonisins B_1 and B_2 in herbal tea and medicinal plants in Turkey by high-performance liquid chromatography. *Journal of Food Protection*, 67(8):1782–1786.

Ono EYS et al. (2002). Post-harvest storage of corn: effect of beginning moisture content on mycoflora and fumonisin contamination. *Food Additives and Contaminants*, 19(11):1081–1090.

Ono EYS et al. (2006). Fumonisins in corn: correlation with *Fusarium* sp. count, damaged kernels, protein and lipid content. *Brazilian Archives of Biology and Technology*, 49:63–71.

Ono EYS et al. (2008a). Mycotoxicological quality evaluation of corn samples used by processing industries in the northern region of Paraná State, Brazil. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 25(11):1392–1399.

Ono E et al. (2008b). Implication of pre-drying steps with natural fumonisin contamination levels in freshly harvested corn. *World Mycotoxin Journal*, 1(3):341–347.

Orsi RB et al. (2007). Effects of oral administration of aflatoxin B_1 and fumonisin B_1 in rabbits (*Oryctolagus cuniculus*). *Chemico-Biological Interactions*, 170(3):201–208.

Orsi RB et al. (2009). Acute toxicity of a single gavage dose of fumonisin B_1 in rabbits. *Chemico-Biological Interactions*, 179:351–355.

Oruc HH, Cengiz M, Kalkanli O (2006). Comparison of aflatoxin and fumonisin levels in maize grown in Turkey and imported from the USA. *Animal Feed Science and Technology*, 128(3–4):337–341.

Osborne CD et al. (2002). Host-resistence to *Trypanosoma cruzi* infection is altered in mice fed *Fusarium moniliforme* (=*F. verticillioides*) culture material. *Food and Chemical Toxicology*, 40:133–142.

Osuchowski MF, Sharma RP (2005). Fumonisin B_1 induces necrotic cell death in BV-2 cells and murine cultured astrocytes and is antiproliferative in BV-2 cells while N2A cells and primary cortical neurons are resistant. *Neurotoxicology*, 26:981–992.

Osuchowski MF, Edwards GL, Sharma RP (2005). Fumonisin B_1–induced neurodegeneration in mice after intracerebroventricular infusion is concurrent with disruption of sphingolipid metabolism and activation of proinflammatory signaling. *Neurotoxicology*, 26:211–221.

Osuchowski MF, He Q, Sharma RP (2005). Endotoxin exposure alters brain and liver effects of fumonisin B_1 in BALB/c mice: implication of blood brain barrier. *Food and Chemical Toxicology*, 43(9):1389–1397.

Oswald IP et al. (2003). The mycotoxin fumonisin B_1 increases intestinal colonization by pathogenic *Escherichia coli* in pigs. *Applied and Environmental Microbiology*, 69:5870–5874.

Oswald IP et al. (2004). In vitro and in vivo effects of fumonisin B_1 on pig lymphocytes: implication for the immune response to vaccination. In: *XI International IUPAC Symposium on Mycotoxins and Phycotoxins, Bethesda, MD, USA*. p. 34.

Pacin A et al. (2009). Fungal and fumonisins contamination in Argentine maize (*Zea mays* L.) silo bags. *Journal of Agricultural and Food Chemistry*, 57:2778–2781.

Paepens C et al. (2004). A flow-through enzyme immunoassay for the screening of fumonisins in maize. *Analytica Chimica Acta*, 523:229–235.

Paepens C et al. (2005a). Evaluation of fumonisin contamination in cornflakes on the Belgian market by "flow-through" assay screening and LC-MS/MS analyses. *Journal of Agricultural and Food Chemistry*, 53(18):7337–7343.

Paepens C et al. (2005b). Development of a liquid chromatography/tandem mass spectrometry method for the quantification of fumonisin B_1, B_2 and B_3 in cornflakes. *Rapid Communications in Mass Spectrometry*, 19:2021–2029.

Pagliuca G et al. (2005). Simple method for the simultaneous isolation and determination of fumonisin B_1 and its metabolite aminopentol-1 in swine liver by liquid chromatography–fluorescence detection. *Journal of Chromatography B*, 818:97–103.

Palencia E et al. (2003). Total fumonisins are reduced in tortillas using the traditional nixtamalization method of Mayan communities. *Journal of Nutrition*, 133(10):3200–3203.

Park JW et al. (2002). Natural co-occurrence of aflatoxin B_1, fumonisin B_1 and ochratoxin A in barley and corn foods from Korea. *Food Additives and Contaminants*, 19(11):1073–1108.

Park JW et al. (2004). Analysis of heat-processed corn foods for fumonisins and bound fumonisins. *Food Additives and Contaminants*, 21:1168–1178.

Park JW et al. (2005). Fungal mycoflora and mycotoxins in Korean polished rice destined for humans. *International Journal of Food Microbiology*, 103(3):305–314.

Parsons MW, Munkvold GP (2010). Associations of planting date, drought stress, and insects with *Fusarium* ear rot and fumonisin B_1 contamination in California maize. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 27(5):591–607.

Pepeljnjak S, Petrik J, Segvic Klaric MS (2005). Toxic effects of *Ustilago maydis* and fumonisin B_1 in rats. *Acta Pharmaceutica (Zagreb, Croatia)*, 55(4):339–348.

Pereira P et al. (2007). Effects of biocontrol agents on *Fusarium verticillioides* count and fumonisin content in the maize agroecosystem: impact on rhizospheric bacterial and fungal groups. *Biological Control*, 42:281–287.

Pereyra C et al. (2010). Fungi and mycotoxins in feed intended for sows at different reproductive stages in Argentina. *Veterinary Medicine International*, 2010, Article ID 569108, 7 pp.

Pewzner-Jung Y et al. (2010a). A critical role for ceramide synthase 2 in liver homeostasis: I. Alterations in lipid metabolic pathways. *Journal of Biological Chemistry*, 285:10 902–10 910.

Pewzner-Jung Y et al. (2010b). A critical role for ceramide synthase 2 in liver homeostasis: II. Insights into molecular changes leading to hepatopathy. *Journal of Biological Chemistry*, 285:10 911–10 923.

Pietri A, Zanetti M, Bertuzzi T (2009). Distribution of aflatoxins and fumonisins in dry-milled maize fractions. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 26(3):372–380.

Piva A et al. (2005). Inability of activated carbon to prevent the toxicity of culture material containing fumonisin B_1 when fed to weaned piglets. *Journal of Animal Science*, 83:1939–1947.

Poling SM, Plattner RD, Weisleder D (2002). *N*-(1-Deoxy-D-fructos-1-yl) fumonisin B_1, the initial reaction product of fumonisin B_1 and D-glucose. *Journal of Agricultural and Food Chemistry*, 50:1318–1324.

Raa H et al. (2009). Glycosphingolipid requirements for endosome-to-Golgi transport of Shiga toxin. *Traffic*, 10:868–882.

Rasmussen RR et al. (2010). Multi-mycotoxin analysis of maize silage by LC-MS/MS. *Analytical and Bioanalytical Chemistry*, 397:765–776.

Reddy RV et al. (1996). Developmental effects of fumonisin B_1 in mice. *Mycopathologia*, 134:161–166.

Reinhold L, Reinhardt K (2011). Mycotoxins in foods in Lower Saxony (Germany): results of official control analyses performed in 2009. *Mycotoxin Research*, 27(2):137–143.

Resnik S, Pacin A, Funes G (2006). [Identification and quantification of mycotoxins in corn harvested in Cordoba Province and in milling products.] In: Rubinstein HR, ed. *Micotoxinas: Impacto en la Producción y Salud Humana y Animal*. Unquillo, Córdoba, Argentina, Narvaja, pp. 199–220 (in Spanish).

Rheeder JP, Marasas WFO, Vismer HF (2002). Production of fumonisins analogs by *Fusarium* species. *Applied and Environmental Microbiology*, 68(5):2101–2105.

Ribeiro DHB et al. (2010). Effects of aflatoxin B_1 and fumonisin B_1 on the viability and induction of apoptosis in rat primary hepatocytes. *International Journal of Molecular Sciences*, 11(4):1944–1955.

Ricci K et al. (2006). Reducing stunting among children: the potential contribution of diagnostics. *Nature*, 444(Suppl. 1):29–38.

Riley RT (2010). The kinetics of urinary fumonisin excretion in humans consuming maize-based foods. *Toxicologist*, 114(Suppl. 1):308–309.

Riley RT, Voss KA (2006). Differential sensitivity of rat kidney and liver to fumonisin toxicity: organ-specific differences in toxin accumulation and sphingoid base metabolism. *Toxicological Sciences*, 92:335–345.

Riley RT, Torres OA, Palencia E (2006). International shipping of fumonisins from maize extracts on C18 sorbent. *Food Additives and Contaminants*, 23:826–832.

Riley RT et al. (2011). Developing mechanism-based and exposure biomarkers for mycotoxins in animals. In: De Saeger S, ed. *Determining mycotoxins and mycotoxigenic fungi in food and feed*. Cambridge, England, Woodhead Publishing Ltd, pp. 245–275.

Rocha L et al. (2009). Mycoflora and co-occurrence of fumonisins and aflatoxins in freshly harvested corn in different regions of Brazil. *International Journal of Molecular Sciences*, 10(11):5090–5103.

Rodrigues I (2008). *BIOMIN mycotoxin survey program 2007.* Herzogenburg, Austria, BIOMIN (http://www.biomin.net).

Rodrigues I, Naehrer K (2011a). BIOMIN mycotoxin survey program 2010. *All About Feed*, 2(2):17–20.

Rodrigues I, Naehrer K (2011b). *BIOMIN mycotoxin report: 1st quarter 2011 results*. Herzogenburg, Austria, BIOMIN (http://www.biomin.net).

Romero-González R et al. (2009). Application of conventional solid-phase extraction for multimycotoxin analysis in beers by ultrahigh-performance liquid chromatography–tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, 57:9385–9392.

Roscoe V et al. (2008). Mycotoxins in breakfast cereals from the Canadian retail market: a 3-year survey. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 25(3):347–355.

Ross PF et al. (1991). Concentrations of fumonisin B_1 in feeds associated with animal health problems. *Mycopathologia*, 114:129–135.

Rudrabhatla M, Wood JS (2007). Rapid, quantitative analysis of multiple mycotoxins by liquid chromatography tandem mass spectrometry. *American Laboratory*, 39:22–25.

Rumora L et al. (2002). Cytotoxic and genotoxic effects of fumonisin B_1 on rabbit kidney RK13 cell line. *Archives of Toxicology*, 76(1):55–61.

Rumora L et al. (2007). Mycotoxin fumonisin B_1 alters cellular redox balance and signaling pathways in rat liver and kidney. *Toxicology*, 242:31–38.

Rychlik M, Asam S (2008). Stable isotope dilution assays in mycotoxin analysis. *Analytical and Bioanalytical Chemistry*, 390:617–628.

Ryu D, Bianchini A, Bullerman LB (2008). Effects of processing on mycotoxins. *Stewart Postharvest Review*, 6(5):1–7.

Sacchi C et al. (2009). Fungal contamination and mycotoxin natural occurrence in oats for race horses feeding in Argentina. *Animal Feed Science and Technology*, 152:330–335.

Sadler TW et al. (2002). Prevention of fumonisin B_1–induced neural tube defects by folic acid. *Teratology*, 66:169–176.

Sakai A et al. (2007). The activities of mycotoxins derived from *Fusarium* and related substances in a short-term transformation assay using v-Ha-ras-transfected BALB/3T3 cells (Bhas 42 cells). *Mutation Research*, 630(1–2):103–111.

Samapundo S et al. (2007). The influence of modified atmospheres and their interaction with water activity on the radial growth and fumonisin B_1 production of *Fusarium verticillioides* and *F. proliferatum* on corn. Part I: The effect of initial headspace carbon dioxide concentration. *International Journal of Food Microbiology*, 114(2):160–167.

Sapsford KE et al. (2006). Rapid detection of foodborne contaminants using an array biosensor. *Sensors and Actuators B*, 113:599–607.

Saunders DS, Meredith FI, Voss KA (2001). Control of fumonisin: effects of processing. *Environmental Health Perspectives*, 109(Suppl. 2):333–336.

Scaff RMC, Scussel VM (2004). Fumonisins B_1 and B_2 in corn-based products commercialized in the state of Santa Catarina—southern Brazil. *Brazilian Archives of Biology and Technology*, 47:911–919.

Schneider E, Usleber E, Märtlbauer E (1995). Rapid detection of fumonisin B_1 in corn-based food by competitive direct dipstick enzyme immunoassay/enzyme-linked immunofiltration assay with integrated negative control reaction. *Journal of Agricultural and Food Chemistry*, 43:2548–2552.

Schneider E et al. (2004). Rapid methods for deoxynivalenol and other trichothecenes. *Toxicology Letters*, 153:113–121.

SCOOP (2003). *Collection of occurrence data of* Fusarium *toxins in food and assessment of dietary intake by the population of EU Member States. Final report.* Brussels, Belgium, European Commission, Directorate-General Health and Consumer Protection, Scientific Cooperation Task 3.2.10 of the European Commission.

Scott PM, Lawrence GA (1992). Liquid chromatographic determination of fumonisins with 4-fluoro-7-nitrobenzofurazan. *Journal of AOAC International*, 75:829–834.

Scott PM, Lawrence GA (1994). Stability and problems in recovery of fumonisins added to corn-based foods. *Journal of AOAC International*, 77:541–545.

Scott PM, Lawrence GA (1996). Determination of hydrolyzed fumonisin B_1 in alkali-processed corn foods. *Food Additives and Contaminants*, 13:823–832.

Scudamore K, Patel S (2008). The fate of deoxynivalenol and fumonisins in wheat and maize during commercial breakfast cereal production. *World Mycotoxin Journal*, 1(4):437–448.

Scudamore K, Patel S (2009). Occurrence of *Fusarium* mycotoxins in maize imported into the UK, 2004–2007. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 26(3):363–371.

Scudamore KA, Scriven F, Patel S (2009). *Fusarium* mycotoxins in the food chain: maize-based snack foods. *World Mycotoxin Journal*, 2(4):441–450.

Scudamore K et al. (2008). Fate of *Fusarium* mycotoxins in maize flour and grits during extrusion cooking. *Food Additives and Contaminants*, 25:1374–1384.

Seefelder W, Knecht A, Humpf H (2003). Bound fumonisin B_1: analysis of fumonisin-B_1 glyco and amino acid conjugates by liquid chromatography–electrospray ionization–tandem mass spectrometry. *Journal of Agricultural and Food Chemistry*, 51:5567–5573.

Segvic Klarić MS et al. (2006). Lipid peroxidation and glutathione levels in porcine kidney PK15 cells after individual and combined treatment with fumonisin B_1, beauvericin and ochratoxin A. *Basic & Clinical Pharmacology & Toxicology*, 100:157–164.

Segvic Klaric MS, Pepeljnjak S, Ruzica R (2008). Genotoxicity of fumonisin B_1, beauvericin and ochratoxin A in porcine kidney PK15 cells: effects of individual and combined treatment. *Croatica Chemica Acta*, 81(1):139–146.

Segvic Klaric MS et al. (2008). Cytotoxicity and apoptosis induced by fumonisin B_1, beauvericin and ochratoxin A in porcine kidney PK15 cells: effects of individual and combined treatment. *Archives of Toxicology*, 82:247–255.

Segvic Klaric M et al. (2009). Co-occurrence of aflatoxins, ochratoxin A, fumonisins, and zearalenone in cereals and feed, determined by competitive direct enzyme-linked immunosorbent assay and thin-layer chromatography. *Archives of Industrial Hygiene and Toxicology*, 60(4):427–434.

Senyuva HZ, Ozcan S (2008). Determination of fumonisins B_1 and B_2 in corn by liquid chromatography/mass spectrometry with immunoaffinity column clean-up: single-laboratory method validation. *Journal of AOAC International*, 91(3):598–606.

Senyuva HZ, Gilbert J, Stroka J (2010). Determination of fumonisins B_1 and B_2 in corn by LC/MS with immunoaffinity column clean-up: interlaboratory study. *Journal of AOAC International*, 93(2):611–621.

Seo E et al. (2009). Fumonisins B_1 and B_2 in agricultural products consumed in South Korea: an exposure assessment. *Journal of Food Protection*, 72(2):436–440.

Sewram V et al. (2003). Fumonisin mycotoxins in human hair. *Biomarkers*, 8(2):110–118.

Sewram V et al. (2005). Production of fumonisin B and C analogues by several *Fusarium* species. *Journal of Agricultural and Food Chemistry*, 53(12):4861–4866.

Sforza S, Dall'Asta C, Marchelli R (2006). Recent advances in mycotoxin determination in food and feed by hyphenated chromatographic techniques/mass spectrometry. *Mass Spectrometry Reviews*, 25:54–76.

Shareef AM (2010). Molds and mycotoxins in poultry feeds from farms of potential mycotoxicosis. *Iraqi Journal of Veterinary Sciences*, 24(1):17–25.

Sharma D et al. (2008). Individual and combined effects of fumonisin B_1 and moniliformin on clinicopathological and cell-mediated immune response in Japanese quail. *Poultry Science*, 87:1039–1051.

Sharma RP et al. (2002). Paradoxical role of tumor necrosis factor alpha in fumonisin-induced hepatotoxicity in mice. *Toxicology*, 180(3):221–232.

Shephard GS, Sewram V (2004). Determination of the mycotoxin fumonisin B_1 in maize by reversed-phase thin-layer chromatography: a collaborative study. *Food Additives and Contaminants*, 21(5):498–505.

Shephard GS, Van der Westhuizen L, Sewram V (2007). Biomarkers of exposure to fumonisin mycotoxins: a review. *Food Additives and Contaminants*, 24:1196–1201.

Shephard GS et al. (1990). Quantitative determination of fumonisins B_1 and B_2 by high-performance chromatography with fluorescence detection. *Journal of Liquid Chromatography*, 13:2077–2087.

Shephard GS et al. (2002a). Fumonisin B_1 in maize harvested in Iran during 1999. *Food Additives and Contaminants*, 19(7):676–679.

Shephard GS et al. (2002b). Preparation of South African maize porridge: effect on fumonisin mycotoxin levels. *South African Journal of Science*, 98:393–396.

Shephard G et al. (2007). Exposure assessment for fumonisins in the former Transkei region of South Africa. *Food Additives and Contaminants*, 24(6):621–629.

Shephard GS et al. (2009). Developments in mycotoxin analysis: an update for 2007–2008. *World Mycotoxin Journal*, 2(1):3–21.

Shephard GS et al. (2010). Developments in mycotoxin analysis: an update for 2008–2009. *World Mycotoxin Journal*, 3(1):3–23.

Shephard GS et al. (2011). Developments in mycotoxin analysis: an update for 2009–2010. *World Mycotoxin Journal*, 4(1):3–28.

Silva LJ et al. (2007). Occurrence of fumonisins B_1 and B_2 in Portuguese maize and maize-based foods intended for human consumption. *Food Additives and Contaminants*, 24(4):381–390.

Silva L et al. (2009). Analysis of fumonisins in corn-based food by liquid chromatography with fluorescence and mass spectrometry detectors. *Food Chemistry*, 112:1031–1037.

Silva LJ et al. (2010). Fumonisin determination in urine by LC-MS-MS. *Analytical and Bioanalytical Chemistry*, 396:809–816.

Simas MMS et al. (2007). Determination of fungal microbiota and mycotoxins in brewers grain used in dairy cattle feeding in the State of Bahia, Brazil. *Food Control*, 18(5):404–408.

Siruguri V, Ganguly C, Bhat R (2009). Utilization of mouldy sorghum and *Cassia tora* through fermentation for feed purposes. *African Journal of Biotechnology*, 8(22):6349–6354.

Smith GW et al. (2002). Cardiovascular changes associated with intravenous administration of fumonisin B_1 in horses. *American Journal of Veterinary Research*, 63:538–545.

Solfrizzo M, De Girolamo A, Visconti A (2001). Determination of fumonisins B_1 and B_2 in cornflakes by high performance liquid chromatography and immunoaffinity clean-up. *Food Additives and Contaminants*, 18(3):227–235.

Solfrizzo M et al. (2004). Comparison of urinary sphingolipids in human populations with high and low maize consumption as a possible biomarker of fumonisin dietary exposure. *Food Additives and Contaminants*, 21:1090–1095.

Songsermsakul P, Razzazi-Fazeli E (2008). A review of recent trends in applications of liquid chromatography–mass spectrometry for determination of mycotoxins. *Journal of Liquid Chromatography and Related Technologies*, 31:1641–1686.

Spanjer MC, Rensen PM, Scholten JM (2008). LC-MS/MS multi-method for mycotoxins after single extraction, with validation data for peanut, pistachio, wheat, maize, cornflakes, raisins and figs. *Food Additives and Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 25(4):472–489.

Speijers GJH, Speijers MHM (2004). Combined toxic effects of mycotoxins. *Toxicology Letters*, 153:91–98.

Stevens VL, Tang J (1997) Fumonisin B_1–induced sphingolipid depletion inhibits vitamin uptake via the glycosylphosphatidylinositol-anchored folate receptor. *Journal of Biological Chemistry*, 272(29):18 020–18 025.

Stockmann-Juvala H et al. (2004a). Fumonisin B_1–induced toxicity and oxidative damage in U-118MG glioblastoma cells. *Toxicology*, 202(3):173–183.

Stockmann-Juvala H et al. (2004b). Oxidative stress and apoptosis in human U-118MG glioblastoma cells exposed to fumonisin B_1. *Toxicology and Applied Pharmacology*, 197(3):334.

Stockmann-Juvala H et al. (2006). Fumonisin B_1–induced apoptosis in neuroblastoma, glioblastoma and hypothalamic cell lines. *Toxicology*, 225(2–3):234–241.

Stockmann-Juvala H et al. (2008). Effects of fumonisin B_1 on the expression of cytokines and chemokines in human dendritic cells. *Food and Chemical Toxicology*, 46(5):1444–1451.

Stoev S et al. (2009). Cytotoxic effect of some mycotoxins and their combinations on human peripheral blood mononuclear cells as measured by the MTT assay. *Open Toxinology Journal*, 2:1–8.

Stoev SD et al. (2010). Mycotic nephropathy in Bulgarian pigs and chickens: complex aetiology and similarity to Balkan endemic nephropathy. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 27:72–88.

Sugita-Konishi Y et al. (2006). Occurrence of aflatoxins, ochratoxin A, and fumonisins in retail foods in Japan. *Journal of Food Protection*, 69(6):1365–1370.

Sulyok M, Krska R, Schuhmacher R (2007a). A liquid chromatography/tandem mass spectrometric multi-mycotoxin method for the quantification of 87 analytes and its application to semi-quantitative screening of moldy food samples. *Analytical and Bioanalytical Chemistry*, 389:1505–1523.

Sulyok M, Krska R, Schuhmacher R (2007b). Application of a liquid chromatography–tandem mass spectrometric method to multi-mycotoxin determination in raw cereals and evaluation of matrix effects. *Food Additives and Contaminants*, 24(10):1184–1195.

Sulyok M, Krska R, Schuhmacher R (2010). Application of an LC-MS/MS based multi-mycotoxin method for the semi-quantitative determination of mycotoxins occurring in different types of food infected by moulds. *Food Chemistry*, 119:408–416.

Sulyok M et al. (2006). Development and validation of a liquid chromatography/tandem mass spectrometric method for the determination of 39 mycotoxins in wheat and maize. *Rapid Communications in Mass Spectrometry*, 20:2649–2659.

Sun G et al. (2007). Fumonisin B_1 contamination of home-grown corn in high-risk areas for esophageal and liver cancer in China. *Food Additives and Contaminants*, 24(2):181–185.

Sun G et al. (2011). Co-contamination of aflatoxin B_1 and fumonisin B_1 in food and human dietary exposure in three areas of China. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 28(4):461–470.

Sydenham EW, Shephard GS, Thiel PG (1992). Liquid chromatographic determination of fumonisins B_1, B_2, and B_3 in foods and feeds. *Journal of the Association of Official Analytical Chemists*, 75:313–318.

Sydenham EW et al. (1996). Liquid chromatographic determination of fumonisins B_1, B_1, and B_3 in corn: AOAC–IUPAC collaborative study. *Journal of AOAC International*, 79:688–696.

Tachampa K et al. (2008). Interactions of organic anion transporters and organic cation transporters with mycotoxins. *Journal of Pharmacological Sciences*, 106(3):435–443.

Takeuchi M, Hamana K, Hiraishi A (2001). Proposal of the genus *Sphingomonas sensu stricto* and three new genera, *Sphingobium*, *Novosphingobium*, and *Sphingopyxis* on the basis of phylogenetic and chemotaxonomic analyses. *International Journal of Systematic and Evolutionary Microbiology*, 51:1405–1417.

Taranu I et al. (2005). Mycotoxin fumonisin B_1 alters the cytokine profile and decreases the vaccinal antibody titer in pigs. *Toxicological Sciences*, 84(2):301–307.

Taranu I et al. (2010). Comparative aspects of in vitro proliferation of human and porcine lymphocytes exposed to mycotoxins. *Archives of Animal Nutrition*, 64(5):383–393.

Tardieu D et al. (2007). Chronic toxicity of fumonisins in turkeys. *Poultry Science*, 86:1887–1893.

Tardieu D et al. (2008). Toxicokinetics of fumonisin B_1 in turkey poults and tissue persistence after exposure to a diet containing the maximum European tolerance for fumonisin in avian feed. *Food and Chemical Toxicology*, 46:3213–3218.

Tardieu D et al. (2009). Tissue persistence of fumonisin B_1 in ducks and after exposure to a diet containing the maximum European tolerance for fumonisins in avian feed. *Chemico-Biological Interactions*, 182:239–244.

Täubel M (2005). *Isolierung und Charakterisierung von Mikroorganismen zur biologischen Inaktivierung von Fumonisinen* [Doctoral Thesis]. Vienna, Austria, University of Natural Resources and Applied Life Sciences [cited in Heinl et al., 2010].

Tessari ENC et al. (2006). Effects of aflatoxin B_1 and fumonisin B_1 on body weight, antibody titres and histology of broiler chicks. *British Poultry Science*, 47(3):357–364.

Tessari ENC et al. (2010). Effects of aflatoxin B_1 and fumonisin B_1 on blood biochemical parameters in broilers. *Toxins*, 2:453–460.

Theumer MG et al. (2002). Immunobiological effects of fumonisin B_1 in experimental subchronic mycotoxicoses in rats. *Clinical and Diagnostic Laboratory Immunology*, 9(1):149–155.

Theumer MG et al. (2003). Immunobiological effects of AFB_1 and AFB_1–FB_1 mixture in experimental subchronic mycotoxicoses in rats. *Toxicology*, 186(1–2):159–170.

Theumer MG et al. (2008). Subchronic mycotoxicoses in rats. Histopathological changes and modulation of the sphinganine to sphingosine (Sa/So) ratio imbalance induced by *Fusarium verticillioides* culture material, due to the coexistence of aflatoxin B_1 in the diet. *Food and Chemical Toxicology*, 46(3):967–977.

Tornyos G et al. (2003). Effect of dietary fumonisin B_1 on certain immune parameters of weaned pigs. *Acta Veterinaria Hungarica*, 51(2):171–179.

Torres OA et al. (2007). Estimated fumonisin exposure in Guatemala is greatest in consumers of lowland maize. *Journal of Nutrition*, 137(12):2723–2729.

Tran ST et al. (2005). Chronic effects of fumonisin B_1 on ducks. *Poultry Science*, 84:22–28.

Trucksess MW et al. (2002). Occurrence of aflatoxins and fumonisins in incaparina from Guatemala. *Food Additives and Contaminants*, 19(7):671–675.

Trung TS et al. (2008). Fungal mycoflora and contamination of maize from Vietnam with aflatoxin B_1 and fumonisin B_1. *World Mycotoxin Journal*, 1:87–94.

Tseng TC, Liu CY (2001). Occurrence of fumonisin B_1 in maize imported into Taiwan. *International Journal of Food Microbiology*, 65(1–2):23–26.

Tumbleson ME et al. (2003). Fumonisin B_1 alters sphinganine and sphingosine concentrations in serum, tissue, urine and cerebrospinal fluid of horses. *Toxicological Sciences*, 72(Suppl. 1):254 (Abstract 1235).

UN (2010). *UNAIDS report on the global AIDS epidemic 2010*. United Nations, Joint United Nations Programme on HIV/AIDS (http://www.unaids.org/globalreport/Global_report.htm, accessed 11 May 2011).

USFDA (2001) *Background paper in support of fumonisin levels in animal feed: executive summary of this scientific support document, November 9, 2001*. Silver Spring, MD, USA, Department of Health and Human Services, Food and Drug Administration (http://www.fda.gov/Food/FoodSafety/FoodContaminantsAdulteration/NaturalToxins/ucm212900.htm).

Vanara F, Reyneri A, Blandino M (2009). Fate of fumonisin B_1 in the processing of whole maize kernels during dry-milling. *Food Control*, 20(3):235–238.

Van der Gaag B et al. (2003). Biosensors and multiple mycotoxin analysis. *Food Control*, 14:251–254.

Van der Westhuizen L et al. (2003). Fumonisin contamination and *Fusarium* incidence in corn from Santa Catarina, Brazil. *Journal of Agricultural and Food Chemistry*, 51(18):5574–5578.

Van der Westhuizen L et al. (2004). Disruption of sphingolipid biosynthesis in hepatocyte nodules: selective proliferation stimulus induced by fumonisin B_1. *Toxicology*, 200:69–75.

Van der Westhuizen L et al. (2008). Sphingoid base levels in humans consuming fumonisin-contaminated maize in rural areas of the former Transkei, South Africa: a cross-sectional study. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 25(11):1385–1391.

Van der Westhuizen L et al. (2010a). Individual fumonisin exposure and sphingoid base levels in rural populations consuming maize in South Africa. *Food and Chemical Toxicology*, 48:1698–1703.

Van der Westhuizen L et al. (2010b). Simple intervention method to reduce fumonisin exposure in a subsistence maize-farming community in South Africa. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 27(11):1582–1588.

Van der Westhuizen L et al. (2011a). Fumonisin B_1 as a urinary biomarker of exposure in a maize intervention study among South African subsistence farmers. *Cancer Epidemiology, Biomarkers & Prevention*, 20:483–489.

Van der Westhuizen L et al. (2011b). Optimising sorting and washing of home-grown maize to reduce fumonisin contamination under laboratory-controlled conditions. *Food Control*, 22:396–400.

Van Egmond HP (2008). [*Research into the presence of fumonisins in 24-h duplicate diets of children*.] Letter report to H.J. Jeuring (0243/2008), VWA. Bilthoven, the Netherlands, National Institute for Public Health and the Environment (RIVM) (in Dutch) [cited in Boon et al., 2009].

Varga J et al. (2010). Fumonisin contamination and fumonisin producing black Aspergilli in dried vine fruits of different origin. International *Journal of Food Microbiology*, 143(3):143–149.

Velluti A, Sanchis V, Ramos A (2004). Effect of essential oils of cinnamon, clove, lemon grass, oregano and palmarosa on growth of and fumonisin B_1 production by *Fusarium verticillioides* in maize. *Journal of the Science of Food and Agriculture*, 84:1141–1146.

Velluti A et al. (2003). Inhibitory effect of cinnamon, clove, lemongrass, oregano and palmarose essential oils on growth and fumonisin B_1 production by *Fusarium proliferatum* in maize grain. *International Journal of Food Microbiology*, 89(2–3):145–154.

Visconti A, Solfrizzo M, De Girolamo A (2001). Determination of fumonisins B_1 and B_2 in corn and corn flakes by liquid chromatography with immunoaffinity column clean-up: collaborative study. *Journal of AOAC International*, 84:1828–1837.

Vishwanath V et al. (2009). Simultaneous determination of 186 fungal and bacterial metabolites in indoor matrices by liquid chromatography/tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 395:1355–1372.

Voss KA, Gelineau-Van Waes JB, Riley RT (2006). Fumonisins: current research trends in developmental toxicology. *Mycotoxin Research*, 22:61–69.

Voss KA, Meredith FI, Bacon CW (2003). Effect of baking and frying on the in vivo toxicity to rats of cornmeal containing fumonisins. *Journal of Agricultural Chemistry*, 51:5546–5551.

Voss KA, Riley RT, Gelineau-Van Waes JB (2007). Fetotoxicity and neural tube defects in CD1 mice exposed to the mycotoxin fumonisin B_1. *Mycotoxins*, 57(Suppl.):67–72.

Voss KA, Riley RT, Gelineau-Van Waes J (2011). Fumonisins. In: Gupta R, ed. *Reproductive and developmental toxicology*. New York, NY, Elsevier, pp. 725–737.

Voss KA, Smith GW, Haschek WM (2007). Fumonisins: toxicokinetics, mechanism of action and toxicity. *Animal Feed Science and Technology*, 137:299–325.

Voss et al. (1990). Comparative studies of hepatotoxicity and fumonisins B_1 and B_2 content of water and chloroform/methanol extracts of *Fusarium moniliforme* strain MRC 826 culture material. *Mycopathologia*, 112:81–92 [cited by Theumer et al., 2002].

Voss KA et al. (1995). Subchronic feeding study of the mycotoxin fumonisin B_1 in B6C3F1 mice and Fischer 344 rats. *Fundamental and Applied Toxicology*, 24:102–110.

Voss KA et al. (2006a). Toxic effects of fumonisin in mouse liver are independent of the peroxisome proliferator-activated receptor α. *Toxicological Sciences*, 89:108–119.

Voss KA et al. (2006b). The role of tumor necrosis factor alpha and the peroxisome proliferator-activated receptor alpha in modulating the effects of fumonisin in mouse liver. *Toxicology*, 222:165–174.

Voss KA et al. (2008). Reduced toxicity of fumonisin B_1 in corn grits by single-screw extrusion with added glucose. *Journal of Food Protection*, 71(10):2036–2041.

Voss KA et al. (2009). Comparing the reproductive and sphingolipid metabolic effects of fumonisin B_1 and its alkaline hydrolysis product in LM/Bc mice: hydrolyzed fumonisin B_1 did not cause neural tube defects. *Toxicological Sciences*, 112:459–467.

Voss KA et al. (2011). Extrusion cooking with glucose supplementation of fumonisin contaminated corn grits protected against nephrotoxicity and disrupted sphingolipid metabolism in rats. *Molecular Nutrition & Food Research*, 55(Suppl. 2):S312–S320.

Wang J, Zhou Y, Wang Q (2008a). Analysis of mycotoxin fumonisins in corn products by high-performance liquid chromatography coupled with evaporative light scattering detection. *Food Chemistry*, 107:970–976.

Wang J et al. (2008b). Fumonisin level in corn-based food and feed from Linxian County, a high-risk area for esophageal cancer in China. *Food Chemistry*, 106(1):241–246.

Wang S et al. (2006). Rapid determination of fumonisin B_1 in food samples by enzyme-linked immunosorbent assay and colloidal gold immunoassay. *Journal of Agricultural and Food Chemistry*, 54:2491–2495.

Whitaker TB et al. (2007). Evaluating the performance of sampling plans to detect fumonisin B_1 in maize lots marketed in Nigeria. *Journal of AOAC International*, 90(4):1050–1059.

WHOSIS (2008). *Children aged <5 years: Indicator definitions and metadata, 2008.* Geneva, Switzerland, World Health Organization, Statistical Information System (http://www.who.int/whosis/indicators/compendium/2008/2nu5/en/index.html, accessed 19 April 2010).

Williams JH et al. (2010). HIV and hepatocellular and esophageal carcinomas related to consumption of mycotoxin-prone foods in sub-Saharan Africa. *American Journal of Clinical Nutrition*, 92(1):154–160.

Williams LD, Meridith FI, Riley RT (2004). Fumonisin-*ortho*-phthalaldehyde derivative is stabilized at low temperature. *Journal of Chromatography B*, 717:135–156.

Wu F, Munkvold GP (2008). Mycotoxins in ethanol co-products: modeling economic impacts on the livestock industry and management strategies. *Journal of Agricultural Chemistry*, 56:3900–3911.

Xu L et al. (2010). Evaluation of fumonisin biomarkers in a cross-sectional study with two high-risk populations in China. *Food Additives & Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*, 27(8):1161–1169.

Yazdanpanah H et al. (2005). Survey of fumonisin B_1 contamination of corn in northern Iran during 2000. *Iranian Journal of Pharmaceutical Research*, 4(4):221–225.

Yazdanpanah H et al. (2006). Human dietary exposure to fumonisin B_1 from Iranian maize harvested during 1998–2000. *Mycopathologia*, 161:395–401.

Zachariasova M et al. (2010). Novel approaches in analysis of *Fusarium* mycotoxins in cereals employing ultra performance liquid chromatography coupled with high resolution mass spectrometry. *Analytica Chimica Acta*, 662:51–61.

Zhang Y et al. (2009). The occurrence and concentration of mycotoxins in US distillers dried grains with solubles. *Journal of Agricultural and Food Chemistry*, 57:9828–9837.

Zinedine A et al. (2006). Natural occurrence of mycotoxins in cereals and spices commercialized in Morocco. *Food Control*, 17(11):868–874.

Zitomer NC et al. (2009). Ceramide synthase inhibition by fumonisin B_1 causes accumulation of 1-deoxy-sphinganine: a novel category of bioactive 1-deoxy-sphingoid bases and 1-deoxy-dihydroceramides biosynthesized by mammalian cell lines and animals. *Journal of Biological Chemistry*, 284:4786–4795.

Zoellner P, Mayer-Helm B (2006). Trace mycotoxin analysis in complex biological and food matrices by liquid chromatography–atmospheric pressure ionisation mass spectrometry. *Journal of Chromatography A*, 1136(2):123–169.

Zomborszky-Kovács M et al. (2002a). Investigations into the time- and dose-dependent effect of fumonisin B_1 in order to determine tolerable limit values in pigs. *Livestock Production Science*, 76(3):251–256.

Zomborszky-Kovács M et al. (2002b). Effects of prolonged exposure to low-dose fumonisin B_1 in pigs. *Journal of Veterinary Medicine. B, Infectious Diseases and Veterinary Public Health*, 49(4):197–201.

APPENDIX 1. FUMONISIN OCCURRENCE TABLES

Table A1-1. Occurrence of fumonisin B_1 in food (non-shaded) and feed (shaded) commodities and their ingredients

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Americas															
Argentina	Entre Rios	Oat grain	2006	17	10	18	88.2		12.5	108	0	42	Sacchi et al. (2009)		
Argentina	Buenos Aires, Entre Rios, Cordoba provinces	Corn puff	2007	20	7		5.0		244.7	928	168		Federico et al. (2010)		
Argentina		Grain maize	2005	100	5		2.0		476	10 812	74.5	746	Sampling under Resolution No. 1075/94 SAGPYA		
Argentina		Grain maize	2006	187	25	50	4.3		520	3653	359	1111			
Argentina		Grain maize	2007	176	89	252	17.6		594	2459	513	1173	Submitted to JECFA		
Argentina	Bragado, Buenos Aires	Milled maize	2008	50	1				660.9				Pereyra et al. (2010)	Yes	Raw materials
Argentina		Soya bean	2008	50	1				82.8	1138					
Argentina	Cordoba, Buenos Aires, Santa Fe provinces	Harvested maize grain	2007	163	10	18	2.4		2374.9	15 495	1280		Pacin et al. (2009)		

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Argentina		Stored maize grain (120–226 days)	2007	163	10	18	1.8		3287.2	23 668	1870				Silo bag
Argentina	Cordoba Province	Corn flour	2005	23	100		75.0		491.3	2600	0	1480	Lerda et al. (2005)		
Argentina		Rice grain	2005	29	100		89.7		89.7	900	0	160			
Argentina	Bragado, Buenos Aires	Pig feed (sow)	2008	50	1				334.2						
Argentina		Pig feed (non-pregnant gilt)	2008	50	1				353.1						Finished feed samples
Argentina		Pig feed (pregnant gilt)	2008	50	1				341.6	512					
Brazil	Sao Paulo State	Infant cereal type C	2004	16	20		0.0		526	1335	480		de Castro et al. (2004)		With corn flour
Brazil		Cornmeal	2004	89	20		0.0		1673	5825	1269				
Brazil		Infant cereal type A-B-D	2004	46	20		100.0								A-B corn starch, D minor corn flour

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil		Corn starch	2004	33	20		100.0								
Brazil		Instant corn-based baby food	2004	12	20		0.0		355	893	289				
Brazil	Sao Paulo State	Cornmeal	2000	30	20		0.0		5170	15 290			Bittencourt et al. (2005)		
Brazil		Corn flour	2000	30	20		0.0		2110	7200					
Brazil	Nine states	Beer	2000–2001	58	0.26 µg/l	1.3 µg/l	56.9		9.6 µg/l	40 µg/l	<LOD	12.6 µg/l	Kawashima, Vieira & Valente Soares (2007)		
Brazil	Paraná State	Freshly harvested maize	2003	100	28		0.0		1810	12 680	1090		Ono et al. (2008b)		
Brazil		Maize industrial delivery posts	2003	200	28		0.0		1830	11 830	1150				
Brazil		Maize before drying	2003	90	28		0.0		2310	10 980	1430				
Brazil	Northern Paraná State	Corn (reception)	2003	300	28		0.0		1730	11 830			Ono et al. (2008a)		
Brazil		Corn (pre-drying)	2003	135	28		0.0		2170	10 980					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil		Corn (reception)	2004	300	28		0.0		1130	12 040					
Brazil		Corn (pre-drying)	2004	135	28		0.0		1180	7740					
Brazil	Northern Paraná State	Freshly harvested maize	2004	100	28		0.0		780	4170			Da Silva et al. (2008)		
Brazil		Maize industrial delivery posts	2004	100	28		0.0		890	3560					
Brazil		Maize before drying	2004	45	28		0.0		1130	7740					
Brazil	Federal District	Cornmeal I (fubà)	2003–2005	62		20		0.0	1240	4740			Caldas & Silva (2007)		
Brazil		Cornmeal II (creme de milho)	2003–2005	11		20		0.0	1430	2560					
Brazil		Precooked flour I (beiju)	2003–2005	21		20		0.0	449	1960					
Brazil		Precooked flour II (milharina)	2003–2005	21		20		0.0	696	1360					

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil		Snacks	2003–2005	20		20		15.0	115	330					
Brazil		Corn flakes	2003–2005	20		20		60.0	108	784					
Brazil		Popcorn	2003–2005	24		20		8.3	398	1240					
Brazil		Sweet corn, on the cob	2003–2005	6		20		100.0							
Brazil		Sweet corn, frozen	2003–2005	8		20		62.5	352	1310					
Brazil		Sweet corn, canned	2003–2005	15		20		80.0	190	1440					
Brazil		Corn	1998–1999	109	100		0.0		3910	8100	3800	5620	Submitted to JECFA		
Brazil		Corn	2003?	88	100		0.0		3234.8	9640	3045	5123			
Brazil	Recife	Canjica (kind of sweet popcorn)	1999–2001	9	2	12	0.0		190	530			Kawashima & Valente Soares (2006)	Yes	White variety of corn
Brazil		Corn flour	1999–2001	10	2	12	16.6		61	150					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil		Corn flakes	1999–2001	31	2	12	0.0		370	870					
Brazil		Cornmeal	1999–2001	11	2	12	9.0		2400	8600					
Brazil		Popcorn	1999–2001	1	2	12	0.0		21	21					
Brazil		Quierera	1999–2001	6	2	12	0.0		410	1400					
Brazil		Quierera fina	1999–2001	6	2	12	0.0		230	400					
Brazil		Corn flakes		20		20		65.0	107	784	<LOQ	422.7			
Brazil		Corn flour (fine)		11		20		0.0	1429.1	2559	1301	2354			
Brazil		Precooked corn flour		42		20		0.0	572.6	1955	392	1170.6			
Brazil		Corn flour		61		20		0.0	1239.3	4736	1032	2039.6			
Brazil		Popcorn		24		20		4.9	398.1	1239	350	755.7			
Brazil		Corn snack		20		20		4.2	114.2	330	76.5	235.6			
Brazil		Sweet corn, frozen		8		20		100.0							

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil		Sweet corn, canned		15		20		80.0	194	1449	<LOQ	788.4			
Brazil	Santa Catarina West	Corn grain	1990–2000	39	10		0.0		2060				Van der Westhuizen et al. (2003)		
Brazil	Santa Catarina North	Corn grain	1990–2000	17	10		0.0		2240						
Brazil	Santa Catarina South	Corn grain	1990–2000	20	10		0.0		1280						
Brazil	Santa Catarina State	Commercial corn flour	2001	25	40		8.0		5017.6	16 455	3825	11 630.6	Scaff & Scussel (2004)		
Brazil		Home-processed corn flour	2001	22	40		9.0		3144.1	15 038	1193	8526			
Brazil		Canjica	2001	12	40		16.6		724	2237	621	1445.9			
Brazil		Corn flakes	2001	11	40		0.0		1139.3	4528	980	1805			
Brazil		Popcorn	2001	12	40		8.3		2336.3	7346	2260	6386			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil	Várzea Grande	Freshly harvested corn	2005	50		15		8.0	730	8440			Rocha et al. (2009)	Yes	
Brazil	Santa Maria	Freshly harvested corn	2005	50		15		0.0	720	6270				Yes	
Brazil	Olivera dos Campinhos	Freshly harvested corn	2005	50		15		0.0	2750	9420				Yes	
Brazil	Nova Odessa	Freshly harvested corn	2005	50		15		0.0	2810	9670				Yes	
Brazil	Rio de Janeiro	Poultry feeds	2003–2004	480	2		2.2			5500			Oliveira et al. (2006)	Yes	
Brazil	Santa Catarina South	Corn grain intended for feeds	1990–2000	14	10		0.0		1050						
Brazil	Rio de Janeiro	Barley rootlets (pig feedstuff)	2004–2005	20	1		0.0		883.1	1383			Cavaglieri et al. (2009)		AFB_1 <LOD (= 1 µg/kg)
Brazil	Bahia State	Grain barley for feed	2007										Batatinha et al. (2007); Simas et al. (2007)	Yes	

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Guatemala		Corn at shops	2009	16	7		0.0		4110	19 340	1590	10 255	Riley, Torres & Palencia (2006)		
Guatemala		Incaparina (mixture of corn and cottonseed flour + vitamins)	2000	3	16		0.0		800	1700	500		Trucksess et al. (2002)	Yes	Corrected by recovery
Guatemala		Maize collected at harvest	2000–2003	396	300		67.2		780				Torres et al. (2007)		
Guatemala		Maize collected from commercial vendors	2005	236	300		38.5		1500						
Guatemala		Maize collected at harvest, highland	2002	83	300		97.6		40						
Guatemala		Maize collected at storage, highland	2002	74	300		73.0		640						

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Uruguay		Corn	2008	3	60		0		18 066		17 662	31 772	Submitted to JECFA		
		Corn	2009	4	60		0		10 148		3717	25 115			
		Corn	2010	10	60		0		3159		1825	5831			
		Barley	2009	3	60		0		149		148	169			
		Barley	2010	26	60		0		219		219	220			
		Wheat	2010	4	60		0		92		92	124			
		Sorghum	2010	4	60		0		151		139	250			
		Malt	2010	6	60		0		280		124				
		Rice	2010	1	60		0		124		124				
USA		Incaparina (mixture of corn and cottonseed flour + vitamins)	1998	5					580	1400	200		Trucksess et al. (2002)	Yes	
USA	Cameron County, Texas	Raw corn	2001	4	25		0		1194	1653	1221	1589.3	de la Campa, Miller & Hendricks (2004)		
USA	Cameron County, Texas	Tortillas	2001	4	25		50		72	175	56	156.4	Dowd & Johnson (2010)		

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Asia															
China	Linxian County	Processed food	2005–2006	39		8	82.1		250	280			Wang et al. (2008)		Mean calculated from positive samples
China	Linxian County	Corn grit	2005–2006	12		8	50.0		230	270					
China	Linxian County	Corn flour	2005–2006	6		8	100.0								
China	Linxian County	Cornmeal	2005–2006	9		8	100.0								
China	Linxian County	Corn tortilla chips	2005–2006	6		8	100.0								
China	Linxian County	Vacuum-packaged cooked corn cob	2005–2006	6		8	83.3		280	280					
China	Linxian County	Unprocessed food	2005–2006	48		8	66.7		470	630					
China	Huaian	Corn	2001–2002	117	16		4.3		2840	25 500			Sun et al. (2007)		
China	Fusui	Corn	2001–2002	94	16		17.0		1270	14 900					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
China	Huantai	Corn	2001–2002	48	16		16.6		650	5700					
China	Shandong Province	Asparagus	2004	30	10		80.0		121	670	65.5	327.8	C. Liu et al. (2005)		
China	Jilin	Corn kernels	2005	45	2		2.2		924	4011	692		Gong et al. (2009)		
China	Henan	Corn kernels	2005	48	2		0.0		935	10 739	316				
China	Hubei	Corn kernels	2005	48	2		0.0		2294	15 158	764				
China	Sichuan	Corn kernels	2005	46	2		0.0		19 837	70 418	9063				
China	Guangxi	Corn kernels	2005	47	2		0.0		11 380	71 121	5623				
China	Guangdong	Corn kernels	2005	48	2		0.0		4888	16 391	3644				
China	Province of Taiwan	Maize	1997–1998	78	40		94.9		31.8	1614			Tseng & Liu (2001)		
China	Province of Taiwan	Maize	1997–1998	20	40		90.0		30.8	334					
China	Province of Taiwan	Maize	1997–1998	10	40		80.0		66.4	477					
China	Province of Taiwan	Maize	1997–1998	10	40		100.0								
China	Province of Taiwan	Corn	2002	20	30		75.0		14.5	70	<LOD	60	F.-M. Liu et al. (2005)		

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
China	Province of Taiwan	Fresh corn	2002	5	30		100.0							
China	Province of Taiwan	Corn snack	2002	15	30		93.3		3.3	50	<LOD			
China	Province of Taiwan	Corn flakes	2002	10	30		100.0							
China	Province of Taiwan	Corn starch	2002	5	30		100.0							
China	Province of Taiwan	Canned corn	2002	5	30		100.0							
China	Province of Taiwan	Corn raw material	2002	16	30		93.3		5.6	90	<LOD			
China	Tibet Autonomous Region, Shigatze Prefecture	Barley	1997									Haubruge et al. (2003)	Yes	
China	Linxian County	Feed	2005–2006	28		8	46.4		1500	3130				
Indonesia		Industrially produced foods	2004									Nuryono et al. (2002)		

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Iraq	Nineveh	Poultry feeds	2005–2007									Shareef (2010)	Yes	
Islamic Republic of Iran	Fars	Corn	2000	15	10		46.7		173	949			Ghiasian et al. (2006)	
Islamic Republic of Iran	Khuzestan	Corn	2000	14	10		42.8		139	732				
Islamic Republic of Iran	Kermanshah	Corn	2000	12	10		58.3		57	342				
Islamic Republic of Iran	Mazandaran Province	Corn	2000	11	10		0.0		7811	11 015				
Islamic Republic of Iran		Maize	2001–2002	4	10		25.0		324				Ghiasian et al. (2009)	
Islamic Republic of Iran		Maize	2001–2002	4	10		25.0		340					
Islamic Republic of Iran		Maize	2001–2002	6	10		34.0		375					

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Islamic Republic of Iran		Maize	2001–2002	3	10		34.0		174						
Islamic Republic of Iran		Maize	2001–2002	10	10		40.0		190						
Islamic Republic of Iran		Maize	2001–2002	6	10		50.0		200						
Islamic Republic of Iran	Mazandaran Province	Maize	2000	38	14		2.7		5670	12 950	5790	9027	Yazdanpanah et al. (2005)		
Islamic Republic of Iran	Mazandaran Province	Maize (at harvest)	1999	20	10		0.0		3175.5	7660	2355	6410	Shephard et al. (2002a)		
Islamic Republic of Iran	Isfahan Province	Maize (markets)	1999	10	10		40.0		220	880	50	610			
Japan		Rice	2004–2005	48	12		100.0						Kushiro et al. (2009)		
Japan		Wheat	2004–2005	47	10		97.8							Only 1 value between LOD and LOQ	

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Raw corn	2004–2007	41		10		100.0					Aoyama et al. (2010)		
Japan		Frozen or canned corn	2004–2007	127		10		98.4	5.3	36					
Japan		Popcorn grain	2004–2007	57		2		14.0	58	354					
Japan		Corn grits	2004–2007	46		2		0.0	104	1380					
Japan		Corn flakes	2004–2007	81		10		81.4	4.6	59					
Japan		Corn soups	2004–2007	88		10		97.7	0.3	12.9					
Japan		Corn starch	2004–2007	22		2		100.0							
Japan		Corn snacks	2004–2007	50		2		18.0	92.7	1670					
Japan		Beer	2004–2007	30		2		66.0	1.6	12.9					
Japan		Buckwheat flour	2004–2007	15		10		100.0							

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Buckwheat dried noodles	2004–2007	50		2		100.0							
Japan		Flattened barley	2004–2007	40		10		100.0							
Japan		Soya beans	2004–2007	82		2		84.1	0.7	8					
Japan		Polished rice	2004–2007	31		4		100.0							
Japan		Millet	2004–2007	30		2		80.0	1	6.5					
Japan		Asparagus	2004–2007	20		2		90.0	0.3	2.8					
Japan		Corn	2007–2008	28	1	2	0.0	0.0	1210.7	4800	885	3130	Submitted to JECFA		
Japan		Corn grits	2004–2009	53	1	2		0.0	146.2	1380	57.6	400.6			
Japan		Raw corn	2004–2009	51	1	10		100.0	<LOD	2.1	<LOD	<LOD			
Japan		Popcorn	2004–2009	69	1	2		23.2	48.8	354	22	117.8			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Sweet corn	2004–2009	126	5	10		98.4	0.5	36	<LOD	<LOD			
Japan		Corn flakes	2004–2009	101	5	10		70.3	7.7	103	<LOD	18			
Japan		Corn soup (liquid)	2004–2009	63	5	10		100.0							
Japan		Corn soup (powder)	2004–2009	46	5	10		95.7	1.2	12.9	<LOD	6.1			
Japan		Corn starch	2004–2009	35	1	2		85.7	2.7	62.7	<LOD	3.5			
Japan		Corn snack	2004–2009	90	1	2		14.4	67.5	1673	35.4	101.8			
Japan		Raw corn	2004–2005	18		10		100.0					Sugita-Konishi et al. (2006)		ND for aflatoxins, OTA
Japan		Canned or frozen corn	2004–2005	51		10		96.0	24.6	36					
Japan		Buckwheat dried noodles	2004–2005	30		2		100.0							
Japan		Popcorn	2004–2005	15		2		0.0	57.2	354					

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Corn flakes	2004–2005	30		10		70.0	27	59					
Japan		Corn soup	2004–2005	29		10		100.0							
Japan		Corn grits	2004–2005	10		2		0.0	51.1	73.8					
Japan		Flattened barley	2004–2005	20		10		100.0							
Japan		Corn feed	2004–2005	48	1	2	0.0		350	1900			Aoyama & Ishikuro (2007)		
Japan		Corn gluten meal (feed)	2004–2005	2	1	2	0.0		130	170					
Japan		Hominy feed	2004–2005	3	1	2	0.0		610	1200					
Japan		Grain sorghum (feed)	2004–2005	11	1	2	0.0		68	340					
Japan		Barley (feed)	2004–2005	14	1	2	35.7		8.1	46					
Japan		Rye (feed)	2004–2005	3	1	2	0.0		16	20					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Wheat feed	2004–2005	2	1	2	50.0		3.6	6.6					
Japan		Dehulled rice (feed)	2004–2005	3	1	2	100.0								
Japan		Soya bean meal (feed)	2004–2005	5	1	2	60.0		<2	3.5					
Japan		Cotton seed (feed)	2004–2005	2	1	2	100.0								
Japan		Alfalfa feed	2004–2005	2	1	2	100.0								
Japan		Beet pulp (feed)	2004–2005	5	1	2	60.0		9.4	41					
Japan		Formula (feed)	2004–2005	9	1	2	0.0		340	780					
Malaysia		Copra cake	2010	3	10		66.7		10	10			Khayoon et al. (2010)		
Malaysia		Pig starter feeder	2010	6	10		83.3		10	10					
Republic of Korea		Corn kernels	2008	78	15	50		17.9	850	9980			Submitted to JECFA		
Republic of Korea		Corn flour	2008	21	15	50		28.6	190	790					

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Republic of Korea		Frozen corn	2008	19	15	50		94.7	10	50					
Republic of Korea		Canned corn	2008	21	15	50		57.1	50	280					
Republic of Korea		Popcorn	2008	12	15	50		91.7	60	710					
Republic of Korea		Corn snack	2008	83	15	50		59.0	130	1090					
Republic of Korea	Seoul	Barley	1998–1999	30	5		100.0						Park et al. (2002)	Yes	
Republic of Korea		Corn	1998–1999	18	5		100.0								
Republic of Korea		Barley foods	1998–1999	32	5		94.0		1.0	16					
Republic of Korea		Corn foods	1998–1999	47	5		81.0		14.2	122					
Republic of Korea	Seoul	Rice	2002	88	35		97.7		1.2	60.6			Park et al. (2005)	Yes	
Republic of Korea	Seoul	Corn flakes	1996	15	5		60.0		35.9	165			Kim et al. (2002)		

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Republic of Korea		Corn snack	1996	14	5		28.6		52.7	188					
Republic of Korea		Corn starch	1996	8	5		62.5		114	332					
Republic of Korea		Corn for popping	1996	12	5		58.3		298	1010					
Republic of Korea		Roasted corn for tea	1996	17	5		100.0								
Republic of Korea		Canned sweet corn	1996	5	5		100.0								
Republic of Korea		Other corn products	1996	5	5		60.0		21.1	24.0					
Republic of Korea		White rice		12	25	37	100.0						Seo et al. (2009)		
Republic of Korea		Brown rice		12	25	37	100.0								
Republic of Korea		Barley		12	25	37	100.0								
Republic of Korea		Barley tea		12	25	37	100.0								
Republic of Korea		Beer		12	25	37	100.0								

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Republic of Korea		Millet		12	25	37	100.0								
Republic of Korea		Wheat flour		12	25	37	100.0								
Republic of Korea		Dried corn		12	25	37	75.0		50.2	268		194.3			
Republic of Korea		Popcorn		12	25	37	100.0								
Republic of Korea		Corn flour		12	25	37	58.3		111.4	440		386.3			
Republic of Korea		Corn tea		12	25	37	100.0								
Republic of Korea		Canned corn		12	25	37	100.0								
Republic of Korea		Breakfast cereal		12	25	37	100.0								
Saudi Arabia	Jeddah	Corn snack (supermarkets)	2009	3	30		100.0						Al-Hazmi (2009)		
Saudi Arabia	Jeddah	Corn snack (supermarkets)	2009	3	30		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Saudi Arabia	Jeddah	Corn snack (supermarkets)	2009	18	30		50.0		640	1850					
Saudi Arabia	Jeddah	Corn snack (supermarkets)	2009	6	30		50.0		1287	1330					
Turkey	Erbeyli	Dried figs	2003	9	46		33.3		142**	495	52**		Karbancioglu-Güler & Heperkan (2009)		
Turkey	Germenincik	Dried figs	2003	21	46		42.9		363**	3200	56**				
Turkey	Incirliova	Dried figs	2003	9	46		22.2		144**	568	78**				
Turkey	Ortaklar	Dried figs	2003	9	46		22.2		324**	1378	91**				
Turkey	Selcuk	Dried figs	2003	13	46		15.4		742**	3649	80**				
Turkey	Soke	Dried figs	2003	7	46		14.3		461**	1804	67**				
Turkey	Torbali	Dried figs	2003	3	46		33.3		143**	335	82**				
Turkey	Erbeyli	Dried figs	2004	6	46		0.0		663**	3153	196**				
Turkey	Germenincik	Dried figs	2004	12	46		25.0		141**	424	110**				
Turkey	Incirliova	Dried figs	2004	4	46		0.0		292**	591	230**				
Turkey	Ortaklar	Dried figs	2004	9	46		22.2		106**	227	69**				
Turkey	Selcuk	Dried figs	2004	6	46		33.3		182**	448	77**				
Turkey	Soke	Dried figs	2004	4	46		25.0		127**	172	157**				

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Turkey	Torbali	Dried figs	2004	3	46		33.3		137**	172	112**				
Turkey	Toplam	Dried figs	2004	44	46		20.5		223	276	115				
Turkey	Istanbul markets	Herbal tea		54	31	103	100.0						Omurtag & Yazicioğlu (2004)		
Turkey	Istanbul markets	Medicinal plants		61	31	103	96.7		823.5	1487					
Turkey		Canned corn	2001	4	1		50.0		155	320	310		Omurtag (2001)		
Turkey		Dried corn (bought from market)	2001	4	1		100.0								
Turkey	Samsun	Dried corn (bought from bazaar)	2001	1	1		100.0								
Turkey	Adana	Dried corn (bought from market)	2001	2	1		100.0								
Turkey	Black Sea	Dried corn (bought from market)	2001	5	1		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Turkey		Dried corn (bought from market)	2001	7	1		85.7		51.4	360	<LOD				
Turkey		Cornmeal (bought from market)	2001	15	1		53.3		284.7	1180	125				
Turkey	Adapazari	Cornmeal (bought from bazaar)	2001	2	1		100.0								
Turkey	Black Sea	Cornmeal (bought from market)	2001	4	1		25.0		540	950	605				
Turkey	Çavusköy	Cornmeal (bought from bazaar)	2001	1	1		100.0								
Turkey	Black Sea	Cornmeal (bought from bazaar)	2001	6	1		50.0		335	1000	62.5				
Turkey	Giresun	Cornmeal (bought from bazar)	2001	2	1		50.0		1330	2660	1330				
Turkey	Samsun	Cornmeal (bought from bazaar)	2001	1	1		100.0								

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Turkey	Samsun	Cornmeal (bought from market)	2001	2	1		50.0		265	530	265				
Turkey		Corn flakes (bought from market)	2001	3	1		100.0								
Turkey		Corn snacks (bought from market)	2001	12	1		83.3		60	370	<LOD				
Turkey	Black Sea	Corn snacks (bought from market)	2001	2	1		100.0								
Turkey		Corn flakes (bought from market)	2001	1	1		100.0								
Turkey		Corn starch (bought from market)	2001	6	1		83.3		43.3	260	<LOD				
Turkey		Popcorn (bought from market)	2001	1	1		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Turkey		Corn bread (bought from market)	2001	1	1		100.0								
Viet Nam	North Viet Nam	Maize (human consumption)	2008	5		100		60.0	350	1150	0	930	Trung et al. (2008)	Yes	
Viet Nam	Central Viet Nam	Maize (human consumption)	2008	3		100		100.0							
Viet Nam	South Viet Nam	Maize (human consumption)	2008	5		100		80.0	100	500	<LOQ	300			
Viet Nam	North Viet Nam	Maize (feed)	2008	5		100		80.0	660	3300	<LOQ	1980			
Viet Nam	Central Viet Nam	Maize (feed)	2008	3		100		100.0							
Viet Nam	South Viet Nam	Maize (feed)	2008	4		100		0.0	855	1120	950	1099			
Oceania															
Australia		Baked beans in tomato sauce composite		4	50		100.0						Submitted to JECFA		

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Australia		Breakfast cereals, mixed grains composite		4	50		100.0							
Australia		Breakfast cereals, single grain composite		4	50		100.0							
Australia		Infant cereal, mixed composite		4	50		100.0							
Australia		Pie, meat, individual size composite		4	50		100.0							
Australia		Sweet corn, kernels, frozen composite		4	50		100.0							
Africa														
Burkina Faso	Kenedougou Province	Stored maize	1999	26	10		0.0		790				Nikièma et al. (2004)	

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Burkina Faso	Kenedougou Province	Maize at harvest	1999	26	10		0.0		120						
Burkina Faso	Banfora	Maize from markets	1999	18	10		0.0		2340						
Burkina Faso	Basi Satiri	Maize from markets	1999	1	10		0.0		1080						
Burkina Faso	Dande	Maize from markets	1999	1	10		0.0		2120						
Burkina Faso	N'Dorola	Maize from markets	1999	13	10		0.0		1380						
Burkina Faso	Kanyan	Maize from markets	1999	3	10		0.0		790						
Burkina Faso	Kourouma	Maize from markets	1999	18	10		0.0		1210						
Burkina Faso		Maize from markets	1999	3	10		0.0		2370						
Burkina Faso		Maize from markets	1999	7	10		7.0		4940						
Cameroon	Bamenda	Maize	2004–2005	12	10		33.3		940	3309			Njobeh et al. (2010)		
Cameroon	Douala	Maize	2004–2005	7	10		0.0		7252	24 225					

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Cameroon	Kumbo	Maize	2004–2005	12	10		58.3		4141	22 502					
Cameroon	Yaounde	Maize	2005	9	10		33.3		2396	5617					
Cameroon	Bamenda	Peanuts	2004–2005	3	10		100.0								
Cameroon	Douala	Peanuts	2004–2005	5	10		80.0		29						
Cameroon	Kumbo	Peanuts	2004–2005	4	10		75.0		25						
Cameroon	Yaounde	Peanuts	2005	4	10		75.0		1498						
Cameroon	Bamenda	Beans	2004–2005	3	10		66.7		764						
Cameroon	Douala	Beans	2004–2005	2	10		100.0								
Cameroon	Kumbo	Beans	2004–2005	10	10		80.0		690	1351					
Cameroon	Bamenda	Soya beans	2004–2005	1	10		100.0								
Cameroon	Douala	Soya beans	2004–2005	2	10		50.0		25						

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Cameroon	Kumbo	Soya beans	2004–2005	2	10		50.0		365						
Congo	Brazaville	Cassava chips		6	1		100.0						Manjula et al. (2009)	Co-occurrence with AFB_1	
Congo	Brazaville	Cassava flour		3	1		100.0							Co-occurrence with AFB_1	
Congo	Brazaville	Maize		2	1		100.0							Co-occurrence with AFB_1	
Congo	Brazaville	White maize		4	1				140	1680				Co-occurrence with AFB_1	
Congo	Brazaville	Mixed maize		4	1				340	9620				Co-occurrence with AFB_1	
Ghana	Accra	Maize from markets	1996	14	50		0.0		608.4	2621			Kpodo, Sorensen & Jakobsen (2000)		
Ghana	Five agroecological zones	Maize from processing sites	1999	75	10		9.3		272.1	1655			Kpodo (2001)	Co-occurrence with aflatoxins	

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Ghana	Accra	Maize from markets	2000	15	10		0.0		435.1	1796					
Ghana	Five agroecological zones	Processed maize (kenkey)	1999	75	10		26.7		202.4	819				Co-occurrence with aflatoxins	
Ghana	Accra	Processed maize (kenkey)	2000	15	10		13.3		244.2	535					
Morocco	Markets in Rabat and Salé	Corn		20	30	60	50.0		1930	5960			Zinedine et al. (2006)	Co-occurrence with OTA and ZEA	
Nigeria	South-western Nigeria	Pre-harvest maize	July–August 2001	103	50		21.4		495	1780	218		Bankole & Mabekoje (2004)	Co-occurrence with aflatoxins	
Nigeria	Ogun State	Stored maize	2000	108	50		49.0		390	1830			Bankole, Mabekoje & Enikuomehin (2003)		

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Nigeria	Niger State	Rice (field samples)	2008	10	10		90.0		0.1	1.0			Makun et al. (2011)	Co-occurrence with aflatoxins, OTA, ZEA, DON	
Nigeria	Niger State	Rice (stored samples)	2008	6	10		66.7		0.8	4.4				Co-occurrence with aflatoxins, OTA, ZEA, DON	
Nigeria	Niger State	Rice (market samples)	2008	5	10		100.0							Co-occurrence with aflatoxins, OTA, ZEA, DON	
South Africa	Mphise and Ngcolosi	Maize		47	50		68.1		2200	22 200			Chelule et al. (2001)		Only FB_1 analysed
South Africa	Durban	Maize		49	50		93.9		300	500					Only FB_1 analysed
South Africa	Mphise and Ngcolosi	Sorghum		13	50		100.0								Only FB_1 analysed

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
South Africa	Mphise and Ngcolosi	Fermented maize meal (amahewu)		14	50		100.0							Only FB_1 analysed
South Africa	Mphise and Ngcolosi	Alcoholic beverage (isizulu)		11	50		100.0							Only FB_1 analysed
South Africa	Mphise and Ngcolosi	Cooked milled maize (phutu)		28	50		71.4		300	400				Only FB_1 analysed
South Africa	Durban	Cooked milled maize (phutu)		39	50		100.0							Only FB_1 analysed
South Africa	KwaZulu Natal	Animal feed		23	5		73.9		1188.8	5900			Mngadi, Govinden & Odhav (2008)	Co-occurrence with aflatoxins, ZEA
Uganda	High altitude (eastern Uganda)	Freshly harvested maize	2007	80			0.0		4930	10 000			Atukwase, Kaaya & Muyanja (2009)	

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Uganda	Mid-altitude (dry)	Freshly harvested maize	2007	80			0.0		4500	8300					
Uganda	Mid-altitude (moist)	Freshly harvested maize	2007	80			0.0		4530	9000					
United Republic of Tanzania	Kilimanjaro	Stored maize	2005	120	53		48.0			6125	206		Kimanya et al. (2008)	Co-occurrence with aflatoxins	Results corrected for recovery
United Republic of Tanzania	Kilimanjaro	Freshly harvested maize	2006	67	20		46.3		1666	12 616	611		Kimanya et al. (2009)		Results corrected for recovery
United Republic of Tanzania	Kilimanjaro	Stored maize	2006	55	20		72.7		333	1349	85				Results corrected for recovery
Zimbabwe		Maize (feed)	2006	1									Submitted to JECFA		
Zimbabwe		Maize (feed)	2006	2	25	80	100.0								
Zimbabwe		Maize (feed)	2007	2											
Zimbabwe		Other feed ingredients	2008	5	25	80	0.0		434.6	1110					

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Zimbabwe		Other feed ingredients	2008	46	25	80	4.3		505.9*	917					
Zimbabwe		Finished feed	2009	11	25	80	36.4		267.6*	726					
Zimbabwe		Finished feed	2009	111	25	80	27.0		494.2*	1890					
Zimbabwe		Silage	2009	6	25	80	0.0		745.2	1060					
Zimbabwe		Silage	2009	2	25	80	100.0								
Zimbabwe		Other feed ingredients	2009	38	25	80	86.8		67*	1457					
Zimbabwe		Cottonseed (feed)	2009	3	25	80	100.0								
Zimbabwe		Maize (feed)	2009	49	25	80	14.3		990.2*	3449					
Zimbabwe		Maize (feed)	2009	2	25	80	50.0		395	395					
Zimbabwe		Cereals (feed)	2009	25	25	80	80		382.1*	7310					
Zimbabwe		Soya bean (feed)	2009	2	25	80	100								
Zimbabwe		Soya bean meal	2009	3	25	80	100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Europe															
Austria		Grains for human consumption	2008	1	20	100		100.0					EFSA		
Austria		Grains for human consumption	2009	3	20	100	100.0								
Austria		Grain milling products	2007	68	20	100	61.8	19.1	221		168	485			
Austria		Grain milling products	2008	12	20	100	50.0	25.0	414		118	1015			
Austria		Grain milling products	2009	10	20	100	100.0								
Austria		Bread and rolls	2008	3	20	100	100.0								
Austria		Pasta (raw)	2008	9	20	100	33.3	44.4	333		332.5	334			
Austria		Breakfast cereals	2007	35	20	100	94.3	5.7							
Austria		Breakfast cereals	2008	6	20	100	16.7	33.3	189		160	260			
Austria		Breakfast cereals	2009	5	20	100	60.0	40.0							

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Austria		Fine bakery wares	2008	2	20	100	50.0	50.0							
Austria		Vegetables and vegetable products (including fungi)	2007	1	20	100	100.0								
Austria		Dried fruits	2007	1	20	100	100.0								
Austria		Beer and beer-like beverages	2008	1	20	100	100.0								
Austria		Cereal-based food for infants	2007	1	20	100	100.0								
Austria		Cereal-based food for infants	2008	1	20	100	100.0								
Austria		Cereal-based food for infants	2008	2	20	100	100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Austria		Other foods	2008	1	20	100	100.0								
Belgium		Grain milling products	2008	10		25		10.0	412		183	1270			
Belgium		Grain milling products	2009	21		25		57.1	113.1		83	321			
Belgium		Grain milling products	2010	32		25		37.5	165.7		77.5	408			
Belgium		Bread and rolls	2008	7		25		85.7	670		670	670			
Belgium		Bread and rolls	2009	12		25		75.0	317		208	700			
Belgium		Bread and rolls	2010	6		25		33.3	34		30	49			
Belgium		Pasta (raw)	2009	11		25		100.0							
Belgium		Pasta (raw)	2010	10		25		70.0	57		58	60			
Belgium		Breakfast cereals	2008	12		25		16.7	86.7		45.5	250			
Belgium		Breakfast cereals	2009	10		25		70.0	83.7		97	116			
Belgium		Breakfast cereals	2010	12		25		41.7	65.7		61	94			

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Belgium		Fine bakery wares	2009	1		25		0.0	239		239	239			
Belgium		Tree nuts	2009	1		25		100.0							
Belgium		Food for infants and small children	2010	4		25		25.0	44		40	53			
Belgium		Cereal-based food for infants and young children	2008	10		25		100.0							
Belgium		Cereal-based food for infants and young children	2009	9		25		100.0							
Belgium		Cereal-based food for infants and young children	2010	6		25		50.0	34.7		36	40			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Belgium		Ready-to-eat meal for infants and young children	2009	1		25		100.0							
Belgium		Products for special nutritional use	2008	1		25		100.0							
Belgium		Products for special nutritional use	2010	1		25		0.0	44		44	44			
Belgium		Vegetable-based meals	2008	1		25		100.0							
Belgium		Snack food	2008	47		25		63.8	132.8		59	470			
Belgium		Snack food	2009	46		25		69.6	104.6		88.5	237			
Belgium		Snack food	2010	50		25		54.0	91.2		59	190			
Belgium	Flemish and Walloon retail stores	Conventional corn flakes	2003–2004	130	20		49.2		82	393	30		Paepens et al. (2005a)		

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Belgium	Flemish and Walloon retail stores	Organic corn flakes	2003–2004	75	20		8.0		144	464	116				
Belgium		Maize	2008	21		25		71.4	71		50	144	EFSA		
Belgium		Maize	2009	15		25		93.3	126		126	126			
Belgium		Maize	2010	15		25		40.0	178.8		79	640			
Belgium		Maize starch	2008	1		25		100.0							
Belgium		Maize starch	2009	2		25		100.0							
Belgium		Maize starch	2010	2		25		50.0	96		96	96			
Bulgaria		Harvested maize	2007	19	24	79	5.3		930	3230			Manova & Mladenova (2009)	Co-occurrence with ZEA	Data corrected for recovery
Cyprus		Grains for human consumption	2007	1	6	20	100.0						EFSA		
Cyprus		Breakfast cereals	2006	1	6	20	100.0								
Cyprus		Fruiting vegetables	2007	10	6	20	100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Czech Republic		Grains as crops	2007	27	100	100	18.5	0.0	364.6		193	839			
Estonia		Oats	2009	1		50	0.0		380.3		380.3	380.3	EFSA		
Estonia		Barley	2009	1		50	0.0		1341.9		1341.9	1341.9			
Estonia		Barley	2010	2	80		100.0								
Estonia		Rye feed	2009	1		50	0.0		1249.9		1249.9	1249.9			
Estonia		Wheat	2009	3		50	0.0		967.5		892.7	1148.1			
Estonia		Compound feedingstuffs	2009	5		50	0.0		1328.8		1493.2	2054.8			
France		Grains as crops	2006	278	71	272	1.4	1.8	2064.2		1198	4424	EFSA		
France		Grains as crops	2007	208	71	272	26.0	39.9	492.2		328	1116			
France		Grains as crops	2008	179	71	272	60.3	32.4	441.6		370	673			
France		Grains as crops	2009	166	71	272	45.2	30.1	685		559	1201			
Germany		Grains and grain-based products	2007	4	7	25	0.0	100.0							

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Grains for human consumption	2001	1	80	280	0.0	100.0							
Germany		Grains for human consumption	2004	5	30	60	0.0	0.0	247.4		237	594.5			
Germany		Grains for human consumption	2005	9	20	40	44.4	0.0	340.1		72	836			
Germany		Grains for human consumption	2006	42	20	40	11.9	14.3	235.7		92.8	560			
Germany		Grains for human consumption	2007	22	20	40	27.3	18.2	115.5		114.4	179			
Germany		Grains for human consumption	2008	52	20	42	34.6	40.4	145.8		64.8	263			
Germany		Grains for human consumption	2009	17	25	25	41.2	29.4	128.7		54	448.9			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Grain milling products	2000	1	20	100	0.0	0.0	220		220	220			
Germany		Grain milling products	2002	12	220	100	41.7	0.0	591.4		460	1230			
Germany		Grain milling products	2003	151	25	50	18.5	17.9	448		95	1504			
Germany		Grain milling products	2004	135	30	60	5.9	8.9	987.6		556	2506			
Germany		Grain milling products	2005	122	30	60	21.3	27.9	293.7		49.9	279.7			
Germany		Grain milling products	2006	156	25	50	12.2	39.1	343.5		64.9	605			
Germany		Grain milling products	2007	115	25	40	23.5	7.8	340.5		120	846.4			
Germany		Grain milling products	2008	264	25	50	29.9	20.1	147.5		84	342.5			
Germany		Grain milling products	2009	214	25	42	46.3	12.6	145.9		82.5	355			
Germany		Bread and rolls	2004	1	9	15	0.0		1365		1365	1365			
Germany		Bread and rolls	2005	3	9	15	0.0		862.3		321	2219			

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Bread and rolls	2006	6	20	40	50.0		1064.5		1384	1774			
Germany		Bread and rolls	2007	13	9	25	30.8	46.2	144.8		43.7	349			
Germany		Bread and rolls	2009	46	20	40	67.4	2.2	83.8		77	130.2			
Germany		Pasta (raw)	2006	18	33	66	5.6	5.6	655.4		645.5	1057			
Germany		Pasta (raw)	2007	44	9	25	54.5	40.9	53.9		53.9	54			
Germany		Pasta (raw)	2009	4	9	15	50.0	0.0	266		266	503			
Germany		Breakfast cereals	2003	85	25	50	24.7	28.2	87.8		60.2	174			
Germany		Breakfast cereals	2004	29	30	60	24.1	20.7	67.6		55.5	144			
Germany		Breakfast cereals	2005	107	30	60	39.3	4.7	41.2		39.4	90.8			
Germany		Breakfast cereals	2006	178	25	50	35.4	29.8	130.9		50.2	322			
Germany		Breakfast cereals	2007	32	20	40	40.6	6.3	46.2		44	87			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Breakfast cereals	2008	58	10	25	15.5	32.8	58.0		35.1	115.5			
Germany		Breakfast cereals	2009	151	25	42	31.1	22.5	92.8		31.2	149.2			
Germany		Breakfast cereals	2010	14	25	25	85.7	0.0	53.5		53.5	69			
Germany		Fine bakery wares	2004	28	30	60	25.0	3.6	320.5		264.5	775			
Germany		Fine bakery wares	2005	65	30	60	12.3	20.0	205.9		40.9	589			
Germany		Fine bakery wares	2006	79	20	40	39.2	22.8	128.8		75.7	297.8			
Germany		Fine bakery wares	2007	68	20	40	11.8	16.2	166.9		109	399			
Germany		Fine bakery wares	2008	61	20	42	34.4	18.0	167.2		190	298.4			
Germany		Fine bakery wares	2009	103	25	50	34.0	37.9	84.2		56	204			
Germany		Fruiting vegetables	2005	2		10	0.0	0.0	11.9		11.9	12.5			
Germany		Fruiting vegetables	2007	13	7	25	84.6	15.4							

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Potatoes and potato products	2008	6	7	25	0.0	100.0							
Germany		Sausages	2007	1	7	25	0.0	0.0	119.9		119.9	119.9			
Germany		Milk and milk product imitations	2006	1	20		100.0	0.0							
Germany		Vegetable oil	2005	3	20	40	100.0	0.0							
Germany		Vegetable oil	2006	1	20	40	100.0	0.0							
Germany		Baking ingredients	2007	1	7	25	0.0	100.0							
Germany		Food for infants and small children	2006	1	10	20	100.0	0.0							
Germany		Infant formula, powder	2006	2	10	20	100.0	0.0							
Germany		Follow-on formula, powder	2003	1		10	0.0	100							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Follow-on formula, powder	2007	1	7	25	100	0.0							
Germany		Follow-on formula, powder	2008	1	10	30	0.0	100.0							
Germany		Follow-on formula, powder	2010	1	25		100.0	0.0							
Germany		Cereal-based food for infants and young children	2002	45	10	20	48.9	48.9	52		52	52			
Germany		Cereal-based food for infants and young children	2004	9	20	20	66.7	0.0	26		27.5	28.9			
Germany		Cereal-based food for infants and young children	2005	1	12	50	100.0	0.0							

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Cereal-based food for infants and young children	2006	90	20	40	37.8	60.0	117		117	138			
Germany		Cereal-based food for infants and young children	2008	11	10	30	27.3	63.6	61.5		61.5	61.5			
Germany		Cereal-based food for infants and young children	2010	12	25		100.0	0.0							
Germany		Ready-to-eat meal for infants and young children	2006	4	20	40	100.0	0.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Ready-to-eat meal for infants and young children	2008	4	10	30	0.0	100.0							
Germany		Products for special nutritional use	2006	1	10	20	100.0	0.0							
Germany		Products for special nutritional use	2009	1	9	15	0.0	0.0	441		441	441			
Germany		Medicinal food[a]	2006	92	13	30	21.7	37.0	553.7		381.9	1403			
Germany		Medicinal food[a]	2007	30	7	25	26.7	20.0	123.6		59.5	423			
Germany		Medicinal food[a]	2008	49	20	42	49.0	6.1	141.2		100.1	347			
Germany		Medicinal food[a]	2009	4	11	42	25.0	25.0	24		24	28			
Germany		Cereal-based dishes	2006	14	20	40	0.0	14.3	335.1		324.5	399			

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Cereal-based dishes	2007	1	10	30	0.0	0.0	342		342	342			
Germany		Cereal-based dishes	2009	1	20	40	100.0	0.0							
Germany		Ready-to-eat soups	2006	2	20		100.0	0.0							
Germany		Snack food	2008	10	5	15	80.0	20.0							
Germany		Ices and desserts	2006	2	10	20	100.0	0.0							
Germany		Grains at harvest	2006	2	7	25			38.8		38.8	52			
Germany		Maize at harvest	2006	44		100		22.7	1910	20 690			Goertz et al. (2010)	Co-occurrence with DON, ZEA	
Germany		Maize at harvest	2007	40		100		100.0						Co-occurrence with DON, ZEA	

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Commercial horsefeed preparations	2007–2008	62	2		6.0		100	2200	27	150	Liesener et al. (2010)	Co-occurrence with DON, ZEA, OTA, ergot alkaloids, T-2 + HT-2	
Hungary		Grain milling products	2004	2	5	20		100.0					EFSA		
Hungary		Grain milling products	2007	2	5	20		50.0	77		77	77			
Hungary		Grain milling products	2008	17	5	20		76.5	247.3		244	422			
Hungary		Grain milling products	2010	2	5	20		0.0	98.5		98.5	153			
Hungary		Bread and rolls	2008	1	5	20		0.0	302		302	302			
Hungary		Breakfast cereals	2008	4	5	20		100.0							
Hungary		Breakfast cereals	2010	1	5	20		0.0	66		66	66			
Hungary		Fine bakery wares	2008	1	5	20		100.0							

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Hungary		Vegetables and vegetable products (including fungi)	2009	1	5	20		100.0							
Hungary		Legumes, beans, green, without pods	2008	1	5	20		100.0							
Hungary		Other fruit products (excluding beverages)	2009	1	5	20		100.0							
Hungary		Snack food	2006	2	5	20		0.0	97		97	131			
Hungary		Snack food	2007	7	5	20		0.0	1067		227	4824			
Hungary		Snack food	2008	1	5	20		0.0	92		92	92			
Hungary		Snack food	2010	1	5	20		0.0	31		31	31			
Hungary		Grains as crops	2006	1	5	20	0.0	0.0	698		698	698			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Hungary		Oat middlings	2008	1	5	20		100.0							
Hungary		Barley	2007	1	5	20		100.0							
Hungary		Barley	2008	1	5	20		100.0							
Hungary		Wheat	2007	2	5	20		100.0							
Hungary		Wheat	2008	2	5	20		100.0							
Hungary		Wheat middlings	2005	1	5	20		0.0	57.0		57	57			
Hungary		Wheat middlings	2006	2	5	20		0.0	172.0		172	213			
Hungary		Wheat middlings	2007	1	5	20		100.0							
Hungary		Wheat middlings	2008	1	5	20		100.0							
Hungary		Spelt	2008	1	5	20		100.0							
Hungary		Maize	2006	2	5	20		100.0							
Hungary		Maize	2007	21	5	20		28.6	439.6		163	1087			
Hungary		Maize	2008	10	5	20		20.0	74.5		68	146			
Hungary		Maize	2009	1	5	20		100.0							

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Hungary		Maize middlings	2004	1	5	20		100.0							
Hungary		Maize middlings	2005	3	5	20		0.0	166.7		160	319			
Hungary		Maize middlings	2006	2	5	20		0.0	39		39	48			
Hungary		Maize middlings	2007	3	5	20		0.0	929.3		1014	1139			
Hungary		Maize middlings	2008	4	5	20		0.0	173		116.5	422			
Hungary		Maize middlings	2009	2	5	20		0.0	169		169	203			
Hungary		Maize middlings	2010	1	5	20		0.0	43		43	43			
Hungary		Maize bran	2008	1	5	20		100.0							
Hungary		Maize gluten	2007	1	5	20		0.0	246		246	246			
Hungary		Malt culms	2007	2	5	20		100.0							
Hungary		Soya (bean), toasted	2007	1	5	20		100.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Hungary		Sunflower seed	2007	1	5	20		100.0							
Hungary		Sunflower seed	2008	1	5	20		100.0							
Hungary		Compound feedingstuffs	2004	3	10	40		100.0							
Hungary		Compound feedingstuffs	2005	14	5	20		21.4	159.6		125	228			
Hungary		Compound feedingstuffs	2006	6	5	20		50.0	392.7		335	532			
Hungary		Compound feedingstuffs	2007	14	5	20		7.1	195.8		126	320			
Hungary		Compound feedingstuffs	2008	7	5	20		42.9	184.5		168	364			
Hungary		Compound feedingstuffs	2009	5	5	20		80.0	68		68	68			
Hungary		Compound feedingstuffs	2010	7	5	20		42.9	91.3		37	265			
Italy	Perugia	Organic corn flour	2007	1		2		0.0	81	81			D'Arco et al. (2009)		
Italy		Organic corn flakes	2007	1		2		100.0							

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Italy		Organic corn products	2007	7		2		14.3	20	39					
Italy		Conventional baby food	2007	9		2		88.9	10	10					
Italy		Conventional corn flour	2007	3		2		33.3	199	235					
Italy		Conventional corn flakes	2007	10		2		100.0							
Italy		Conventional pasta	2007	14		2		85.7	5	7					
Italy		Conventional cookies	2007	11		2		81.8	5	7					
Italy		Conventional corn products	2007	26		2		80.8	37	126					
Italy		Corn flakes	2001	8		5		12.5	265.6	1092		Solfrizzo, De Girolamo & Visconti (2001)			
Italy		Mixed cereals	2001	3		5		0.0	184	290					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Italy		Crunchy nut	2001	1		5		0.0	15					
Italy		Miel pops	2001	1		5		0.0	145					
Italy		Cruscam	2001	1		5		0.0	176					
Italy		Cao croks	2001	1		5		0.0	14					
Italy		Sugar coated	2001	1		5		0.0	26					
Italy		Choco	2001	1		5		0.0	22					
Italy		Mixed cereals and cocoa	2001	1		5		0.0	25					
Italy		Raw cereals (maize, wheat barley, farro, rice, etc.)	March 2001 – February 2002	111	10		68.0			2870	70		Cirillo et al. (2003a)	Co-occurrence with DON data available
Italy		Bread, toast, special breads	March 2001 – February 2002	24	10		71.0			150	50			Co-occurrence with DON data available

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Italy		Durum wheat pasta	March 2001 – February 2002	17	10		100.0							Co-occurrence with DON data available	
Italy		Breakfast cereals (corn flakes, muesli, etc.)	March 2001 – February 2002	14	10		50.0			350	71			Co-occurrence with DON data available	
Italy		Biscuits	March 2001 – February 2002	24	10		92.0			200				Co-occurrence with DON data available	
Italy		Baby and infant foods	March 2001 – February 2002	12			100.0							Co-occurrence with DON data available	
Italy		Pig liver		3	0	10			22.1				Gazzotti et al. (2011)		No HFB_1 or HFB_2 detected

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Italy		Pig liver		3	0	10			15.8						No HFB_1 detected
Italy		Pig liver		3	0	10			42.5						No HFB_1 detected
Italy		Pig liver		3	0	10			35.6						No HFB_1 detected
Italy		Pig liver		3	0	10			Traces						No HFB_1 detected
Italy		Pig liver		3	0	10			24.2						No HFB_1 detected
Italy		Pig liver		3	0	10			Traces						HFB_1 detected at level of 17.36 µg/kg
Italy	Apulia market	Red wine	2004–2008										Logrieco et al. (2010)		FB_4 analysed for but not detected
Italy	Apulia market	White wine	2004–2008												FB_4 analysed for but not detected
Italy	Apulia market	Rosé wine	2004–2008												FB_4 analysed for but not detected

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Italy		Feed	January 2005 – August 2009	28									Griessler et al. (2010)	Co-occurrence with DON, ZEA, aflatoxins, OTA	
Lithuania		Breakfast cereals	2009	1	6	19		100.0					EFSA		
Lithuania		Breakfast cereals	2010	2	6	19		50.0	156		156	156			
Lithuania		Barley	2009	1	6	19		0.0	931		931	931	EFSA		
Lithuania		Maize	2009	4	6	19		50.0	67.5		67.5	90			
Lithuania		Maize	2010	4	6	19		0.0	58.8		49	92			
Lithuania		Compound feedingstuffs	2009	2	6	19		0.0	5720		5720	6800			
Lithuania		Compound feedingstuffs	2010	1	6	19		100.0							
Luxembourg		Grains for human consumption	2009	4		60	0.0	50.0	121.5		121.5	154.0	EFSA		

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Luxembourg		Grain milling products	2009	12	20	60	8.3	33.3	277.5		211.3	616.2			
Luxembourg		Pasta (raw)	2009	4		60	0.0	25.0	230.8		152.4	388.7			
Luxembourg		Breakfast cereals	2009	20	20	60	45.0	35.0	114.5		114.0	144.9			
Netherlands		Vegetable oil	2005	1	100		100.0						EFSA		
Netherlands		Vegetable oil	2008	1	100		100.0								
Netherlands		Feed	2005	7	100		100.0								
Netherlands		Feed	2006	1	100		100.0								
Netherlands		Feed	2009	2	100		100.0								
Netherlands		Cereal grains, their products and by-products	2004	1	100		100.0								
Netherlands		Cereal grains, their products and by-products	2005	3	100		100.0								
Netherlands		Cereal grains, their products and by-products	2006	3	100		100.0								

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Cereal grains, their products and by-products	2007	1	100		100.0								
Netherlands		Cereal grains, their products and by-products	2008	7	100		85.7		380		380	380			
Netherlands		Cereal grains, their products and by-products	2009	3	100		100.0								
Netherlands		Oats	2003	2	100		100.0								
Netherlands		Oats	2006	4	100		100.0								
Netherlands		Oats	2007	4	100		100.0								
Netherlands		Oats	2008	8	100		100.0								
Netherlands		Oats	2009	6	100		100.0								
Netherlands		Barley	2003	7	100		100.0								
Netherlands		Barley	2004	14	100		100.0								
Netherlands		Barley	2005	8	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Barley	2006	8	100		100.0								
Netherlands		Barley	2007	11	100		100.0								
Netherlands		Barley	2008	2	100		100.0								
Netherlands		Barley	2009	17	100		100.0								
Netherlands		Millet	2005	1	100		100.0								
Netherlands		Millet	2006	2	100		100.0								
Netherlands		Millet	2007	1	100		100.0								
Netherlands		Millet	2008	1	100		100.0								
Netherlands		Millet	2009	2	100		100.0								
Netherlands		Rye middlings	2006	1	100		100.0								
Netherlands		Sorghum	2006	1	100		100.0								
Netherlands		Sorghum	2007	7	100		85.7		130		130	130			
Netherlands		Sorghum	2008	3	100		66.7		180		180	180			
Netherlands		Wheat	2003	9	100		100.0								
Netherlands		Wheat	2004	23	100		100.0								
Netherlands		Wheat	2005	16	100		100.0								
Netherlands		Wheat	2006	22	100		100.0								
Netherlands		Wheat	2007	11	100		100.0								

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Wheat	2008	6	100		100.0								
Netherlands		Wheat	2009	11	100		90.9		140		140	140			
Netherlands		Wheat middlings	2003	3	100		100.0								
Netherlands		Wheat middlings	2004	4	100		100.0								
Netherlands		Wheat middlings	2005	4	100		100.0								
Netherlands		Wheat middlings	2006	1	100		100.0								
Netherlands		Wheat middlings	2007	3	100		100.0								
Netherlands		Wheat middlings	2009	2	100		100.0								
Netherlands		Wheat feed	2004	1	100		100.0								
Netherlands		Wheat feed	2005	1	100		100.0								
Netherlands		Wheat feed	2006	1	100		100.0								
Netherlands		Wheat feed	2007	4	100		100.0								
Netherlands		Wheat bran	2006	1	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Wheat bran	2007	1	100		100.0								
Netherlands		Wheat germ	2006	1	100		100.0								
Netherlands		Wheat gluten feed	2003	1	100		100.0								
Netherlands		Wheat gluten feed	2004	1	100		100.0								
Netherlands		Wheat gluten feed	2006	1	100		100.0								
Netherlands		Wheat starch	2005	1	100		100.0								
Netherlands		Spelt	2009	1	100		100.0								
Netherlands		Triticale	2003	4	100		100.0								
Netherlands		Triticale	2004	1	100		100.0								
Netherlands		Triticale	2005	2	100		100.0								
Netherlands		Triticale	2006	2	100		100.0								
Netherlands		Triticale	2007	3	100		100.0								
Netherlands		Triticale	2008	1	100		100.0								
Netherlands		Triticale	2009	1	100		100.0								
Netherlands		Maize	2003	29	100		82.8		4202		1700	11 000			
Netherlands		Maize	2004	62	100		83.9		692		585	1400			

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Maize	2005	67	100		88.1		976.3		725	2900			
Netherlands		Maize	2006	57	100		87.7		267.1		160	660			
Netherlands		Maize	2007	87	100		55.2		1853.6		1000	4800			
Netherlands		Maize	2008	79	100		78.5		2804.7		2500	4400			
Netherlands		Maize	2009	38	100		92.1		156.7		150	190			
Netherlands		Maize bran	2005	1	100		100.0								
Netherlands		Maize germ expeller	2008	1	100		0.0		120		120	120			
Netherlands		Maize gluten feed	2003	4	100		0.0		3000		2650	5000			
Netherlands		Maize gluten feed	2004	10	100		10.0		687.8		570	1700			
Netherlands		Maize gluten feed	2005	17	100		17.6		287.1		255	460			
Netherlands		Maize gluten feed	2006	13	100		46.2		3945.7		4400	6900			
Netherlands		Maize gluten feed	2007	7	100		14.3		7100		6500	15 000			
Netherlands		Maize gluten feed	2008	2	100		0.0		1245		1245	2200			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Maize gluten feed	2009	2	100		50.0		1300		1300	1300			
Netherlands		Malt culms	2007	1	100		100.0								
Netherlands		Brewers' dried grains	2005	2	100		50.0		530		530	530			
Netherlands		Brewers' dried grains	2006	4	100		50.0		485		485	790			
Netherlands		Brewers' dried grains	2009	10	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2003	8	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2004	19	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2005	21	100		100.0								

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Oil seeds, oil fruits, their products and by-products	2006	24	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2007	19	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2008	13	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2009	29	100		100.0								
Netherlands		Rape seed	2004	5	100		100.0								
Netherlands		Rape seed	2005	1	100		100.0								
Netherlands		Rape seed	2006	3	100		100.0								
Netherlands		Rape seed	2007	4	100		100.0								
Netherlands		Rape seed	2009	2	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Rape seed, expeller	2008	4	100		100.0								
Netherlands		Rape seed, expeller	2009	8	100		100.0								
Netherlands		Rape seed, extracted	2003	5	100		80.0		200		200	200			
Netherlands		Rape seed	2004	7	100		100.0								
Netherlands		Rape seed	2005	6	100		100.0								
Netherlands		Rape seed	2007	4	100		100.0								
Netherlands		Rape seed	2008	6	100		100.0								
Netherlands		Rape seed	2009	7	100		100.0								
Netherlands		Palm kernel expeller	2003	9	100		100.0								
Netherlands		Palm kernel expeller	2004	8	100		100.0								
Netherlands		Palm kernel expeller	2005	18	100		100.0								
Netherlands		Palm kernel expeller	2006	10	100		100.0								
Netherlands		Palm kernel expeller	2007	12	100		100.0								

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Palm kernel expeller	2008	12	100		100.0								
Netherlands		Palm kernel expeller	2009	6	100		100.0								
Netherlands		Palm kernel, extracted	2005	1	100		100.0								
Netherlands		Palm kernel, extracted	2008	1	100		100.0								
Netherlands		Soya (bean), toasted	2003	3	100		100.0								
Netherlands		Soya (bean), toasted	2004	3	100		100.0								
Netherlands		Soya (bean), toasted	2006	1	100		100.0								
Netherlands		Soya (bean), toasted	2008	2	100		100.0								
Netherlands		Soya (bean), extracted, toasted	2003	14	100		100.0								
Netherlands		Soya (bean)	2004	33	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Soya (bean)	2005	51	100		100.0								
Netherlands		Soya (bean)	2006	54	100		100.0								
Netherlands		Soya (bean)	2007	39	100		97.4		440		440	440			
Netherlands		Soya (bean)	2008	43	100		95.3		575		575	610			
Netherlands		Soya (bean)	2009	61	100		98.4		110		110	110			
Netherlands		Niger seed expeller	2009	1	100		100.0								
Netherlands		Sunflower seed	2003	2	100		100.0								
Netherlands		Sunflower seed	2004	3	100		100.0								
Netherlands		Sunflower seed	2005	4	100		100.0								
Netherlands		Sunflower seed	2006	4	100		100.0								
Netherlands		Sunflower seed	2007	1	100		100.0								
Netherlands		Sunflower seed	2009	4	100		100.0								
Netherlands		Sunflower seed, extracted	2003	3	100		100.0								

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Sunflower seed, extracted	2004	7	100		100.0								
Netherlands		Sunflower seed, extracted	2005	4	100		100.0								
Netherlands		Sunflower seed, extracted	2006	14	100		100.0								
Netherlands		Sunflower seed, extracted	2007	10	100		100.0								
Netherlands		Sunflower seed, extracted	2008	5	100		100.0								
Netherlands		Sunflower seed, extracted	2009	9	100		100.0								
Netherlands		Sunflower seed, partially decorticated, extracted	2008	1	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Linseed	2003	1	100		100.0								
Netherlands		Linseed	2004	1	100		100.0								
Netherlands		Linseed	2005	1	100		100.0								
Netherlands		Linseed	2006	1	100		100.0								
Netherlands		Linseed	2007	2	100		100.0								
Netherlands		Linseed expeller	2003	1	100		100.0								
Netherlands		Linseed expeller	2007	1	100		100.0								
Netherlands		Linseed expeller	2008	2	100		100.0								
Netherlands		Cocoa husks	2003	2	100		100.0								
Netherlands		Sweet lupins	2003	1	100		100.0								
Netherlands		Sweet lupins	2008	1	100		100.0								
Netherlands		Peas	2003	1	100		100.0								
Netherlands		Peas	2004	4	100		100.0								
Netherlands		Peas	2005	4	100		100.0								
Netherlands		Peas	2006	3	100		100.0								
Netherlands		Peas	2008	2	100		100.0								

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Tubers, roots, their products and by-products	2004	9	100		100.0								
Netherlands		Tubers, roots, their products and by-products	2005	1	100		100.0								
Netherlands		Tubers, roots, their products and by-products	2006	2	100		100.0								
Netherlands		Tubers, roots, their products and by-products	2007	4	100		100.0								
Netherlands		Tubers, roots, their products and by-products	2008	5	100		100.0								
Netherlands		(Sugar) beet pulp	2003	7	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		(Sugar) beet pulp	2004	11	100		100.0								
Netherlands		(Sugar) beet pulp	2005	19	100		94.7		110		110	110			
Netherlands		(Sugar) beet pulp	2006	11	100		100.0								
Netherlands		(Sugar) beet pulp	2007	1	100		100.0								
Netherlands		(Sugar) beet pulp	2008	1	100		100.0								
Netherlands		Sweet potato	2006	1	100		100.0								
Netherlands		Potato pulp	2003	1	100		100.0								
Netherlands		Potato pulp	2005	1	100		100.0								
Netherlands		Potato pulp	2006	2	100		100.0								
Netherlands		Potato starch	2003	1	100		100.0								
Netherlands		Potato protein	2005	1	100		100.0								
Netherlands		Potato protein	2006	1	100		100.0								

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Other seeds and fruits, their products and by-products	2003	1	100		100.0								
Netherlands		Other seeds and fruits, their products and by-products	2004	2	100		100.0								
Netherlands		Other seeds and fruits, their products and by-products	2006	1	100		100.0								
Netherlands		Carob pods	2008	1	100		100.0								
Netherlands		Citrus pulp	2003	9	100		100.0								
Netherlands		Citrus pulp	2004	22	100		95.5		800		800	800			
Netherlands		Citrus pulp	2005	15	100		100.0								
Netherlands		Citrus pulp	2006	1	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Citrus pulp	2007	2	100		100.0								
Netherlands		Citrus pulp	2008	6	100		100.0								
Netherlands		Citrus pulp	2009	5	100		100.0								
Netherlands		Forages and roughage	2008	1	100		100.0								
Netherlands		Forages and roughage	2003	127	100		66.9		672.4		515	1400			
Netherlands		Forages and roughage	2004	166	100		71.1		415.6		235	610			
Netherlands		Forages and roughage	2005	168	100		90.5		190		145	330			
Netherlands		Forages and roughage	2006	171	100		68.4		276.9		255	440			
Netherlands		Forages and roughage	2007	75	100		81.3		530.7		260	1800			
Netherlands		Forages and roughage	2008	97	100		71.1		528.9		450	1200			
Netherlands		Forages and roughage	2009	76	100		93.4		686		790	1400			
Netherlands		Lucerne meal	2003	1	100		100.0								

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Lucerne meal	2005	2	100		100.0							
Netherlands		Lucerne meal	2006	7	100		100.0							
Netherlands		Lucerne meal	2007	14	100		100.0							
Netherlands		Lucerne meal	2008	2	100		100.0							
Netherlands		Clover meal	2006	1	100		100.0							
Netherlands		Grass meal	2003	2	100		100.0							
Netherlands		Grass meal	2004	3	100		100.0							
Netherlands		Grass meal	2005	12	100		100.0							
Netherlands		Grass meal	2006	3	100		100.0							
Netherlands		Grass meal	2007	19	100		100.0							
Netherlands		Grass meal	2008	20	100		100.0							
Netherlands		Grass meal	2009	2	100		100.0							
Netherlands		Other plants, their products and by-products	2008	2	100		100.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Milk products	2005	4	100		75.0		1200		1200	1200			
Netherlands		Milk products	2006	1	100		100.0								
Netherlands		Whey powder	2005	1	100		100.0								
Netherlands		Poultry meal	2003	13	100		76.9		293.3		270	430			
Netherlands		Poultry meal	2004	50	100		66.0		452.4		280	870			
Netherlands		Poultry meal	2005	36	100		75.0		1216.7		320	7200			
Netherlands		Poultry meal	2008	2	100		0.0		1800		1800	2200			
Netherlands		Poultry meal	2009	2	100		100.0								
Netherlands		Minerals	2006	1	100		0.0		170		170	170			
Netherlands		Minerals	2008	1	100		100.0								
Netherlands		Bakery and pasta products and by-products	2006	1	100		100.0								
Netherlands		Bakery and pasta products and by-products	2003	1	100		100.0								

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Bakery and pasta products and by-products	2004	5	100		100.0								
Netherlands		Bakery and pasta products and by-products	2005	10	100		100.0								
Netherlands		Bakery and pasta products and by-products	2006	14	100		100.0								
Netherlands		Bakery and pasta products and by-products	2007	1	100		100.0								
Netherlands		Bakery and pasta products and by-products	2009	1	100		100.0								
Netherlands		Confectionery products and by-products	2004	1	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Compound feedingstuffs	2004	8	100		50.0		225		215	330			
Netherlands		Compound feedingstuffs	2005	6	100		66.7		235		235	330			
Netherlands		Compound feedingstuffs	2006	2	100		100.0								
Netherlands		Compound feedingstuffs	2008	1	100		0.0		300		300	300			
Netherlands		Compound feedingstuffs	2009	6	100		66.7		130		130	150			
Portugal		Yellow maize	2005	9	20		33.0		322	871			Silva et al. (2007)		
Portugal		White maize	2005	2	20		0.0		363	725					
Portugal		Maize flour	2005	3	20		33.0		822	1569					
Portugal		Maize semolina	2005	3	20		33.0		118	183					
Portugal		Maize starch	2005	3	20		100.0								
Portugal		Sweet maize	2005	11	20		82.0		64	523					
Portugal		Corn flakes	2005	16	20		100.0								
Portugal		Mixed cereals	2005	4	20		100.0								

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Portugal		Maize snacks	2005	16	20		93.8		10.6	171					
Portugal	Coimbra	Maize bread (broa)	2005	30	20		20.0		197	448	205		Lino et al. (2007)		
Portugal	Local retail markets	Cornmeal		41	20		0.0		474.3	1300			Martins et al. (2008)		
Portugal	Local retail markets	Sweet corn (popcorn)		49	20		26.6		153.9	400					
Portugal	Local retail markets	Corn flakes		15	20		100.0								
Portugal		Mixed feed for laying hens		52	50		80.8		73.6	110			Martins, Guerra & Bernardo (2006)	Co-occurrence with ZEA and DON	
Portugal		Soya (feed raw materials)	2000–2007	26	20		100.0						Martins et al. (2008)	Co-occurrence with AFB_1 and DON	
Portugal		Rice (feed raw materials)	2000–2007	17	20		94.1		15					Co-occurrence with AFB_1 and DON	

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Portugal		Corn (feed raw materials)	2000–2007	58	20		79.3			300				Co-occurrence with AFB_1 and DON	
Portugal		Wheat (feed raw materials)	2000–2007	50	20		92.0			40				Co-occurrence with AFB_1 and DON	
Portugal		Barley (feed raw materials)	2000–2007	29	20		93.1		10					Co-occurrence with AFB_1 and DON	
Portugal		Ensilage	2000–2007	13	20		100.0							Co-occurrence with AFB_1 and DON	
Portugal		Gluten	2000–2007	15	20		100.0							Co-occurrence with AFB_1 and DON	
Slovakia		Grains for human consumption	2008	2	20	50		100.0					EFSA		

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Slovakia		Grains for human consumption	2009	9	20	50		44.4	137.1		144.3	188.7			
Slovakia		Grain milling products	2008	4	20	50		25.0	185		186.9	266			
Slovakia		Grain milling products	2009	17	20	50		82.4	505.1		470	962.2			
Slovakia		Bread and rolls	2008	2	20	50		100.0							
Slovakia		Bread and rolls	2009	11	20	50		72.7	87.5		83.0	110.1			
Slovakia		Breakfast cereals	2009	2	15	44		50.0	97		97	97			
Slovakia		Fine bakery wares	2009	4	15	44		100.0							
Slovakia		Fruiting vegetables	2008	5	15	44		0.0	115.6		81.0	262.4			
Slovakia		Fruiting vegetables	2009	2	20	50		100.0							
Slovakia		Snack food	2008	1	20	50		100.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Slovakia		Snack food	2009	12	20	50		75.0	144		156	210			
Slovakia		Grains as crops	2008	1	15	44		0.0	56.7		56.7	56.7			
Slovakia		Grains as crops	2009	3	20	50		33.3	77.5		77.5	103.0			
Spain		Grains for human consumption	2008	7		100	0.0	100.0					EFSA		
Spain		Grains for human consumption	2009	6		100	0.0	100.0							
Spain		Grains for human consumption	2010	7		100	0.0	100.0							
Spain		Grain milling products	2009	8		200	0.0	87.5	1106						
Spain		Grain milling products	2010	5		60	0.0	100.0							
Spain		Bread and rolls	2009	12		200	0.0	100.0							

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Spain		Bread and rolls	2010	5		200	0.0	100.0							
Spain		Pasta (raw)	2008	4		100	0.0	100.0							
Spain		Pasta (raw)	2009	4		100	0.0	100.0							
Spain		Pasta (raw)	2010	4		100	0.0	100.0							
Spain		Breakfast cereals	2008	1		100	0.0	100.0							
Spain		Breakfast cereals	2009	10		200	0.0	100.0							
Spain		Breakfast cereals	2010	3		60	0.0	100.0							
Spain		Fine bakery wares	2009	6		200	0.0	100.0							
Spain		Fine bakery wares	2010	5		200	0.0	100.0							
Spain		Cereal-based food for infants and young children	2008	6		100	0.0	100.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Spain		Cereal-based food for infants and young children	2009	16		200	0.0	100.0							
Spain		Ready-to-eat meal for infants and young children	2009	1		200	0.0	100.0							
Spain		Snack food	2009	10		200	0.0	70.0	516.3	922	533	649			
Spain		Snack food	2010	3		60	0.0	100.0							
Spain	Valencia	Corn flour	2006	9	12	35	44.4		455	922			Silva et al. (2009)		
Spain		Sweet corn	2006	6	12	35	100.0								
Spain		Corn snacks	2006	9	12	35	88.9		68	68					
Spain		Corn flakes	2006	11	12	35	100.0								
Spain		Bread and rolls	2006	3	12	35	100.0								

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Spain	Valencia	Organic baby food	2007	5		2		40.0	156	449			D'Arco et al. (2009)		
Spain		Organic corn flakes	2007	1		2		0.0	3	3					
Spain		Organic cookies	2007	1		2		0.0	40	40					
Spain		Organic corn products	2007	4		2		25.0	12	25					
Spain		Conventional baby food	2007	48		2		93.8	3	3					
Spain		Conventional corn flour	2007	7		2		28.6	18	45					
Spain		Conventional corn flakes	2007	11		2		81.8	18	30					
Spain		Conventional cookies	2007	5		2		60.0	56	91					
Spain		Conventional corn products	2007	22		2		86.4	34	50					
Spain	Aragón	Conventional corn grain	2001–2003	30		25		86.7	43.2**	354			Ariño, Estopañan & González-Cabo (2007)		

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Spain	Aragón	Organic corn grain	2001–2003	30		25		90.0	35.4**	359					
Europe		Other feed ingredients	2006	3	25	80	100.0						Submitted to JECFA		
Europe		Other feed ingredients	2006	3	25	80	66.7		1030	1030					
Europe		Other feed ingredients	2006	3	25	80	100.0								
Europe		Maize (feed)	2006	4	25	80	0.0		198	442					
Europe		Maize (feed)	2006	1	25	80	100.0								
Europe		Soya bean	2006	2	25	80	100.0								
Europe		Maize (feed)	2007	5	25	80	0.0		1582.8*	4518					
Europe		Other feed ingredients	2007	5	25	80	0.0		739.4	2356					
Europe		Other feed ingredients	2007	4	25	80	0.0		541	586					
Europe		Other feed ingredients	2008	27	25	80	0.0		1486.2	7992					
Europe		Other feed ingredients	2008	4	25	80	50.0		240.5	271					

Table A1-1 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Europe		Finished feed	2008	3	25	80	66.7		64	64					
Europe		Finished feed	2008	6	25	80	33.3		1280.7*	4058					
Europe		Maize (feed)	2009	6	25	80	0.0		2778.5	6065					
Europe		Maize (feed)	2009	6	25	80	0.0		1713.5	3297					
Europe		Maize (feed)	2009	8	25	80	50.0		227.5*	655					
Europe		Cereal	2009	13	25	80	69.2		294.6*	1639					
Europe		Other feed ingredients	2009	6	25	80	100.0								
Europe		Finished feed	2009	16	25	80	6.2		284.8*	570					
Europe		Finished feed	2009	3	25	80	33.3		794.5	882					
Europe		Finished feed	2009	4	25	80	100.0								
Europe		Finished feed	2009	2	25	80	100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_1 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Europe		Finished feed	2009	2	25	80	0.0		834.5	1170					
Europe		Breeder layer mash	2009	2	25	80	0.0		476	736					

AFB_1, aflatoxin B_1; DON, deoxynivalenol; EFSA, European Food Safety Authority; LOD, limit of detection; LOQ, limit of quantification; ND, not detected; OTA, ochratoxin A; SAGPYA, Secretaría de Agricultura, Ganadería, Pesca y Alimentos de la Nación, Argentina; ZEA, zearalenone

* = Mean of all samples where non-detects are assigned a value of zero.

** = Mean or median calculated using one half the LOQ or LOD for results lower than the LOQ or LOD.

[a] Specially formulated and intended for the dietary management of a disease that has distinctive nutritional needs that cannot be met by normal diet alone; intended to be used under medical supervision.

Table A1-2. Occurrence of fumonisin B_2 in food (unshaded) and feed (shaded) commodities and their ingredients

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Americas															
Argentina	Buenos Aires, Entre Rios, Cordoba provinces	Corn puff	2007	20	30		30.0		56.3	435	23.5		Federico et al. (2010)		
Argentina		Grain maize	2005	100	5		19.0		157	4967	33	330	Sampling under Resolution No. 1075 /94 SAGPYA		
Argentina		Grain maize	2006	187	25	50	18.2		233.8	1581.5	126.8	670.5			
Argentina		Grain maize	2007	176	50	142	47.7		118	579	<LOD	272.8	Submitted to JECFA		
Argentina	Cordoba, Buenos Aires, Santa Fe provinces	Harvested maize grain	2007	163	6	30	7.9		704.3	4902	356		Pacin et al. (2009)		
Argentina		Stored maize grain (120–226 days)	2007	163	6	30	3.6		951.3	7440	551				Silo bag
Argentina	Cordoba Province	Corn flour	2005	23	100		82.6		52.2	500	<LOD	180	Lerda et al. (2005)		

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Argentina		Rice grain	2005	29	100		100.0								
Brazil	Sao Paulo State	Infant cereal type C	2004	16	20		37.5		95	307	89		de Castro et al. (2004)		With corn flour
Brazil		Cornmeal	2004	89	20		0.0		415	1687	308				
Brazil		Infant cereal type A-B-D	2004	46	20		100.0								A-B corn starch, D minor corn flour
Brazil		Corn starch	2004	33	20		100.0								
Brazil		Instant corn-based baby food	2004	12	20		41.7		53	142	50				
Brazil	Sao Paulo State	Cornmeal	2000	30	30		0.0		1000	3940			Bittencourt et al. (2005)		
Brazil		Corn flour	2000	30	30		0.0		670	1760					
Brazil	Paraná State	Maize freshly after harvesting	2003	100	35		16.0		840	5260	550		Ono et al. (2008b)		
Brazil		Maize industrial delivery posts	2003	200	35		16.0		740	5250	590				

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil		Maize before drying	2003	90	35		36.0		1250	7890	800				
Brazil	Northern Paraná State	Corn (reception)	2003	300	35		26.3		670	5225			Ono et al. (2008a)		
Brazil		Corn (pre-drying)	2003	135	35		32.6		1000	7890					
Brazil		Corn (reception)	2004	300	35		46.0		560	6120					
Brazil		Corn (pre-drying)	2004	135	35		43.0		560	3470					
Brazil	Northern Paraná State	Maize freshly after harvesting	2004	100	35		32.0		320	1040			Da Silva et al. (2008)		
Brazil		Maize industrial delivery posts	2004	100	35		49.0		480	2000					
Brazil		Maize before drying	2004	45	35		38.0		730	3470					
Brazil	Federal District	Cornmeal I (fubà)	2003–2005	62		20			439	1570			Caldas & Silva (2007)		

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil		Cornmeal II (creme de milho)	2003–2005	11		20			617	1090					
Brazil		Precooked flour I (beiju)	2003–2005	21		20			204	534					
Brazil		Precooked flour II (milharina)	2003–2005	21		20			397	1020					
Brazil		Snacks	2003–2005	20		20			64	260					
Brazil		Corn flakes	2003–2005	20		20			19	122					
Brazil		Popcorn	2003–2005	24		20			266	858					
Brazil		Sweet corn, on the cob	2003–2005	6		20			<LOQ	<LOQ					
Brazil		Sweet corn, frozen	2003–2005	8		20			<LOQ	<LOQ					
Brazil		Sweet corn, canned	2003–2005	15		20			<LOQ	<LOQ					
Brazil		Corn	2003	88	100		1.1		2686.7	7540	2210	5006	Submitted to JECFA		

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil		Corn flakes		20		20		60.0	18.1	122	0	78.8			
Brazil		Corn flour (fine)		11		20		0.0	616.6	1086	537	895			
Brazil		Precooked corn flour		42		20		2.4	299.9	1017	219	536.3			
Brazil		Corn flour		61		20		0.0	439.1	1513	348.5	734.5			
Brazil		Popcorn		24		20		8.3	266.5	858	260	526.9			
Brazil		Corn snack		20		20		35.0	62	252	21	203.4			
Brazil		Sweet corn, frozen		8		20		50.0	70.9	201	28	170.2			
Brazil		Sweet corn, canned		15		20		100.0							
Brazil	Santa Catarina West	Corn grain	1990–2000	39	18		0.0		790				Van der Westhuizen et al. (2003)		
Brazil	Santa Catarina North	Corn grain	1990–2000	17	18		0.0		910						
Brazil	Santa Catarina South	Corn grain	1990–2000	20	18		0.0		350						

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil	Santa Catarina State	Commercial corn flour	2001	25	50		40.0		1340.1	5466	464	4317.3	Scaff & Scussel (2004)		
Brazil		Home-processed corn flour	2001	22	50		54.5		667.4	4184	<LOD	3002.3			
Brazil		Canjica	2001	12	50		91.6		8.2	98	<LOD	<LOD			
Brazil		Corn flakes	2001	11	50		72.7		138.6	1328	<LOD	1085			
Brazil		Popcorn	2001	12	50		50.0		535.8	2427	158.5	2039.5			
Brazil	Várzea Grande	Freshly harvested corn	2005	50		15		44.0	190	3030			Rocha et al. (2009)	Yes	
Brazil	Santa Maria	Freshly harvested corn	2005	50		15		50.0	150	1180				Yes	
Brazil	Olivera dos Campinhos	Freshly harvested corn	2005	50		15		2.0	620	1310				Yes	
Brazil	Nova Odessa	Freshly harvested corn	2005	50		15		6.0	950	3160				Yes	

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil	Santa Catarina South	Corn grain intended for feeds	1990–2000	14	18		0.0		370				Oliveira et al. (2006)		
Guatemala		Corn at shops	2009	16	7		0.0		1080	7260	300	2500	Riley, Torres & Palencia (2006)		
Guatemala		Incaparina (mixture of corn and cottonseed flour + vitamins)	2000	3	16		100.0						Trucksess et al. (2002)	Yes	Corrected by recovery
Uruguay		Corn	2008		104				7475.3		7330	13 226	Submitted to JECFA		
Uruguay		Corn	2009		104				4113.3		1943	9800.3			
Uruguay		Corn	2010		104				1274.2		829.5	2660			
Uruguay		Barley	2009		104				269.7		277	277			
Uruguay		Barley	2010		104				150.2		141	165			
Uruguay		Wheat	2010		104				123		104	157.2			
Uruguay		Sorghum	2010		104				132.3		122.5	168.3			
Uruguay		Malt	2010		104				154.3		160.5	180			
Uruguay		Rice	2010		104				180						

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
USA		Incaparina (mixture of corn and cottonseed flour + vitamins)	1998	5					200	600	150		Trucksess et al. (2002)	Yes	
Asia															
China	Shandong Province	Asparagus	2004	30	5		80.0		35.1	138	31.5	56.1	C. Liu et al. (2005)		
China	Province of Taiwan	Corn	2002	20	70		85.0		12	90	<LOD	71	F.-M. Liu et al. (2005)		
China	Province of Taiwan	Fresh corn	2002	5	70		80.0		30	150	<LOD	90			
China	Province of Taiwan	Corn snack	2002	15	70		93.3		10.7	160	<LOD	<LOD			
China	Province of Taiwan	Corn flakes	2002	10	70		100.0								
China	Province of Taiwan	Corn starch	2002	5	70		100.0								
China	Province of Taiwan	Canned corn	2002	5	70		100.0								
China	Province of Taiwan	Corn raw material	2002	16	70		100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
China	Tibet Autonomous Region, Shigatze Prefecture	Barley	1997										Haubruge et al. (2003)	Yes	
Islamic Republic of Iran	Fars	Corn	2000	15	10		86.7		33	306			Ghiasian et al. (2006)		
Islamic Republic of Iran	Khuzestan	Corn	2000	14	10		78.6		28	329					
Islamic Republic of Iran	Kermanshah	Corn	2000	12	10		83.3		11	106					
Islamic Republic of Iran	Mazandaran Province	Corn	2000	11	10		0.0		2158	3364					
Islamic Republic of Iran		Maize	2001–2002	4	10				<LOD				Ghiasian et al. (2009)		
Islamic Republic of Iran		Maize	2001–2002	4	10				53						

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Islamic Republic of Iran		Maize	2001–2002	6	10				22						
Islamic Republic of Iran		Maize	2001–2002	3	10				<LOD						
Islamic Republic of Iran		Maize	2001–2002	10	10				<LOD						
Islamic Republic of Iran		Maize	2001–2002	6	10				<LOD						
Japan		Rice	2004–2005	48	11		100.0						Kushiro et al. (2009)		
Japan		Wheat	2004–2005	47	8		100.0								Only 1 value between LOD and LOQ
Japan		Raw corn	2004–2007	41		10		100.0					Aoyama et al. (2010)		
Japan		Frozen or canned corn	2004–2007	127		10		99.2	0.1	14.8					

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Popcorn grain	2004–2007	57		2		22.8	13.8	94				
Japan		Corn grits	2004–2007	46		2		8.7	33.3	590				
Japan		Corn flakes	2004–2007	81		10		100.0						
Japan		Corn soups	2004–2007	88		10		100.0						
Japan		Corn starch	2004–2007	22		2		100.0						
Japan		Corn snacks	2004–2007	50		2		20.0	25.4	597				
Japan		Beer	2004–2007	30		2		100.0						
Japan		Buckwheat flour	2004–2007	15		10		100.0						
Japan		Buckwheat dried noodles	2004–2007	50		2		100.0						
Japan		Flattened barley	2004–2007	40		10		100.0						

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Soya beans	2004–2007	82		2		96.3	0.2	4.8					
Japan		Polished rice	2004–2007	31		4		100.0							
Japan		Millet	2004–2007	30		2		100.0							
Japan		Asparagus	2004–2007	20		2		90.0	0.3	2.5					
Japan		Corn	2007–2008	28	1	2		0.0	463.2	1900	270	1230	Submitted to JECFA		
Japan		Corn grits	2004–2009	53	0.6	2		5.7	42.2	590	17.6	96.5			
Japan		Raw corn	2004–2009	51	1	10		100.0	<LOD	<1	<LOD	<LOD			
Japan		Popcorn	2004–2009	69	1	2		23.2	11.5	94	6	28.2			
Japan		Sweet corn	2004–2009	126	5	10		99.2	0.2	15	<LOD	<LOD			
Japan		Corn flakes	2004–2009	101	5	10		99.0	0.4	18.9	<LOD	<LOD			
Japan		Corn soup (liquid)	2004–2009	63	5	10		100.0	<LOD	<LOD	<LOD	<LOD			

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Corn soup (powder)	2004–2009	46	5	10		100.0	<LOD	<LOD	<LOD	<LOD			
Japan		Corn starch	2004–2009	35	1	2		91.4	1.2	16.7	<LOD	1.8			
Japan		Corn snack	2004–2009	90	1	2		17.8	18.5	597	7	28.8			
Japan		Raw corn	2004–2005	18		10		100.0					Sugita-Konishi et al. (2006)		ND for aflatoxins, OTA
Japan		Canned or frozen corn	2004–2005	51		10		98.0	14.8	14.8					
Japan		Buckwheat dried noodles	2004–2005	30		2		100.0							
Japan		Popcorn	2004–2005	15		2		6.7	16.9	94					
Japan		Corn flakes	2004–2005	30		10		100.0							
Japan		Corn soup	2004–2005	29		10		100.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Corn grits	2004–2005	10		2		0.0	21.1	29.1					
Japan		Flattened barley	2004–2005	20		10		100.0							
Japan		Corn feed	2004–2005	48	1	2	0.0		140	880			Aoyama & Ishikuro (2007)		
Japan		Corn gluten meal (feed)	2004–2005	2	1	2	0.0		110	130					
Japan		Hominy feed	2004–2005	3	1	2	0.0		220	390					
Japan		Grain sorghum (feed)	2004–2005	11	1	2	9.0		20	91					
Japan		Barley (feed)	2004–2005	14	1	2	35.0		3.4	14					
Japan		Rye (feed)	2004–2005	3	1	2	0.0		7.3	9					
Japan		Wheat feed	2004–2005	2	1	2	50.0		<LOQ	3.1					
Japan		Dehulled rice (feed)	2004–2005	3	1	2	100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Soya bean meal (feed)	2004–2005	5	1	2	80.0		<LOQ	2.9					
Japan		Cotton seed (feed)	2004–2005	2	1	2	100.0								
Japan		Alfalfa feed	2004–2005	2	1	2	100.0								
Japan		Beet pulp (feed)	2004–2005	5	1	2	60.0		3.8	15					
Japan		Formula (feed)	2004–2005	9	1	2	0.0		130	250					
Malaysia		Copra cake	2010	3	40		100.0						Khayoon et al. (2010)		
Malaysia		Pig starter feed	2010	6	40		100.0						Khayoon et al. (2010)		
Republic of Korea		Corn kernels	2008	78	30	100		66.7	140	1740			Submitted to JECFA		
Republic of Korea		Corn flour	2008	21	30	100		90.5	20	150					
Republic of Korea		Frozen corn	2008	19	30	100		100.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (μg/kg)	LOQ (μg/kg)	% < LOD	% < LOQ	Mean (μg/kg)	Maximum (μg/kg)	Median (μg/kg)	90th percentile (μg/kg)			
Republic of Korea		Canned corn	2008	21	30	100		100.0							
Republic of Korea		Popcorn	2008	12	30	100		91.7	10	130					
Republic of Korea		Corn snack	2008	83	30	100		92.8	20	180					
Republic of Korea		White rice		12	25	37	100.0						Seo et al. (2009)		
Republic of Korea		Brown rice		12	25	37	100.0								
Republic of Korea		Barley		12	25	37	100.0								
Republic of Korea		Barley tea		12	25	37	100.0								
Republic of Korea		Beer		12	25	37	100.0								
Republic of Korea		Millet		12	25	37	100.0								
Republic of Korea		Wheat flour		12	25	37	100.0								
Republic of Korea		Dried corn		12	25	37	100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Republic of Korea		Popcorn		12	25	37	100.0								
Republic of Korea		Corn flour		12	25	37	100.0								
Republic of Korea		Corn tea		12	25	37	100.0								
Republic of Korea		Canned corn		12	25	37	100.0								
Republic of Korea		Breakfast cereal		12	25	37	100.0								
Thailand	Chiangmai and Chumporn provinces	*Coffea arabica* and canephora beans	2006–2007	96	1	1	93.7		0.2	9.7		0	Noonim et al. (2009)	1 positive FB_4	Screening ELISA-HPLC-MS/MS
Turkey	Istanbul markets	Herbal tea		54	468	1562	100.0						Omurtag & Yazicioğlu (2004)		
Turkey	Istanbul markets	Medicinal plants		61	468	1562	100.0								
Turkey		Canned corn	2001	4	5		100.0						Omurtag (2001)		

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (μg/kg)	LOQ (μg/kg)	% < LOD	% < LOQ	Mean (μg/kg)	Maximum (μg/kg)	Median (μg/kg)	90th percentile (μg/kg)			
Turkey		Dried corn (bought from market)	2001	4	5		100.0								
Turkey	Samsun	Dried corn (bought from bazaar)	2001	1	5		100.0								
Turkey	Adana	Dried corn (bought from market)	2001	2	5		100.0								
Turkey	Black Sea	Dried corn (bought from market)	2001	5	5		100.0								
Turkey		Dried corn (bought from market)	2001	7	5		100.0								
Turkey		Cornmeal (bought from market)	2001	15	5		93.3		36.7	550	<LOD				
Turkey	Adapazari	Cornmeal (bought from bazaar)	2001	5	5		100.0								
Turkey	Black Sea	Cornmeal (bought from market)	2001	4	5		100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Turkey	Çavusköy	Cornmeal (bought from bazaar)	2001	1	5		100.0								
Turkey	Black Sea	Cornmeal (bought from bazaar)	2001	6	5		100.0								
Turkey	Giresun	Cornmeal (bought from bazaar)	2001	2	5		100.0								
Turkey	Samsun	Cornmeal (bought from bazaar)	2001	1	5		100.0								
Turkey	Samsun	Cornmeal (bought from market)	2001	3	5		100.0								
Turkey		Corn flakes (bought from market)	2001	3	5		100.0								
Turkey		Corn snacks (bought from market)	2001	12	5		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Turkey	Black Sea	Corn snacks (bought from market)	2001	2	5		100.0							
Turkey		Corn flakes (bought from market)	2001	1	5		100.0							
Turkey		Corn starch (bought from market)	2001	6	5		100.0							
Turkey		Popcorn (bought from market)	2001	1	5		100.0							
Turkey		Corn bread (bought from market)	2001	1	5		100.0							
Oceania														
Australia		Baked beans in tomato sauce composite		4	50		100.0					Submitted to JECFA		
Australia		Breakfast cereals, mixed grains composite		4	50		100.0							

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Australia		Breakfast cereals, single grain composite		4	50		100.0							
Australia		Infant cereal, mixed composite		4	50		100.0							
Australia		Pie, meat, individual size composite		4	50		100.0							
Australia		Sweet corn, kernels, frozen composite		4	50		100.0							
Africa														
Burkina Faso	Kenedougou Province	Stored maize	1999	26	10		0.0		380				Nikièma et al. (2004)	
Burkina Faso	Kenedougou Province	Maize at harvest	1999	26	10		53.8		20					
Burkina Faso	Banfora	Maize from markets	1999	18	10		0.0		1050					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Burkina Faso	Basi Satiri	Maize from markets	1999	1	10		0.0		420						
Burkina Faso	Dande	Maize from markets	1999	1	10		0.0		790						
Burkina Faso	N'Dorola	Maize from markets	1999	13	10		0.0		420						
Burkina Faso	Kanyan	Maize from markets	1999	3	10		0.0		440						
Burkina Faso	Kourouma	Maize from markets	1999	18	10		5.5		540						
Burkina Faso		Maize from markets	1999	3	10		0.0		1090						
Burkina Faso		Maize from markets	1999	7	10		0.0		1900						
Ghana	Accra	Maize from markets	1996	14	50		21.4		235.4	1375			Kpodo, Sorensen & Jakobsen (2000)		
Ghana	Five agroecological zones	Maize from processing sites	1999	75	10		30.6		129.6	772			Kpodo (2001)	Co-occurrence with aflatoxins	

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Ghana	Accra	Maize from markets	2000	15	10		13.3		162.2	513					
Ghana	Five agroecological zones	Processed maize (kenkey)	1999	75	10		62.7		38.7	186				Co-occurrence with aflatoxins	
Ghana	Accra	Processed maize (kenkey)	2000	15	10		26.7		38.1	68					
Nigeria	South-western Nigeria	Pre-harvest maize	July–August 2001	103	50		34.0		114	230	72		Bankole & Mabekoje (2004)	Co-occurrence with aflatoxins	
Nigeria	Niger State	Rice (field samples)	2008	10	20		90.0		13.2	132.5			Makun et al. (2011)	Co-occurrence with aflatoxins, OTA, ZEA, DON	

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Nigeria	Niger State	Rice (stored samples)	2008	6	20		100.0							Co-occurrence with aflatoxins, OTA, ZEA, DON	
Nigeria	Niger State	Rice (market samples)	2008	5	20		100.0							Co-occurrence with aflatoxins, OTA, ZEA, DON	
United Republic of Tanzania	Kilimanjaro	Freshly harvested maize	2006	67	20		49.2		1097	9050	362		Kimanya et al. (2009)		Results corrected for recovery
United Republic of Tanzania	Kilimanjaro	Stored maize	2006	55	20		87.2		278	576	249				Results corrected for recovery
Zimbabwe		Other feed ingredients	2008	5	25	80	0.0		248.2	489			Submitted to JECFA		
Zimbabwe		Other feed ingredients	2008	46	25	80		6.5	199*	368					
Zimbabwe		Finished feed	2009	11	25	80	36.4		154.9*	455					

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Zimbabwe		Finished feed	2009	111	25	80	36.0		198.8*	784					
Zimbabwe		Silage	2009	6	25	80	0.0		245	347					
Zimbabwe		Silage	2009	2	25	80	100.0								
Zimbabwe		Other feed ingredients	2009	38	25	80	94.7		22.7*	578					
Zimbabwe		Cottonseed (feed)	2009	3	25	80	100.0								
Zimbabwe		Maize (feed)	2009	49	25	80	22.4		307.8*	949					
Zimbabwe		Maize (feed)	2009	2	25	80	50.0		297	297					
Zimbabwe		Cereals (feed)	2009	25	25	80	84.0		169.2*	3175					
Zimbabwe		Soya bean (feed)	2009	2	25	80	100.0								
Zimbabwe		Soya bean meal	2009	3	25	80	100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Europe															
Austria		Grains for human consumption	2008	1	20	100	100.0	0.0					EFSA		
Austria		Grains for human consumption	2009	3	20	100	100.0	0.0							
Austria		Grain milling products	2007	68	20	100	91.2	5.9	128.5		128.5	156			
Austria		Grain milling products	2008	12	20	100	83.3	8.3	247		247	247			
Austria		Grain milling products	2009	10	20	100	100.0	0.0							
Austria		Bread and rolls	2008	3	20	100	100.0	0.0							
Austria		Pasta (raw)	2008	9	20	100	77.8	22.2							
Austria		Breakfast cereals	2007	35	20	100	100.0	0.0							
Austria		Breakfast cereals	2008	6	20	100	66.7	33.3							
Austria		Breakfast cereals	2009	5	20	100	100.0	0.0							

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Austria		Fine bakery wares	2008	2	20	100	100.0	0.0							
Austria		Vegetables and vegetable products (including fungi)	2007	1	20	100	100.0	0.0							
Austria		Dried fruits	2007	1	20	100	100.0	0.0							
Austria		Beer and beer-like beverages	2008	1	20	100	100.0	0.0							
Austria		Cereal-based food for infants	2007	1	20	100	100.0	0.0							
Austria		Cereal-based food for infants	2008	1	20	100	100.0	0.0							
Austria		Cereal-based food for infants	2008	2	20	100	100.0	0.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Austria		Other foods	2008	1	20	100	100.0	0.0							
Belgium		Grain milling products	2008	10		50		40.0	144.2		116	331			
Belgium		Grain milling products	2009	21		50		100.0							
Belgium		Grain milling products	2010	32		50		78.1	73.7		66	113			
Belgium		Bread and rolls	2008	7		50		85.7	107						
Belgium		Bread and rolls	2009	12		50		83.3	99.5		99.5	144			
Belgium		Bread and rolls	2010	6		50		100.0							
Belgium		Pasta (raw)	2009	11		50		100.0							
Belgium		Pasta (raw)	2010	10		50		80.0	76.5		76.5	79			
Belgium		Breakfast cereals	2008	12		50		100.0							
Belgium		Breakfast cereals	2009	10		50		100.0							
Belgium		Breakfast cereals	2010	12		50		100.0							

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Belgium		Fine bakery wares	2009	1		50		100.0						
Belgium		Tree nuts	2009	1		50		100.0						
Belgium		Food for infants and small children	2010	4		50		100.0						
Belgium		Cereal-based food for infants and young children	2008	10		50		100.0						
Belgium		Cereal-based food for infants and young children	2009	9		50		100.0						
Belgium		Cereal-based food for infants and young children	2010	6		50		100.0						

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Belgium		Ready-to-eat meal for infants and young children	2009	1		50		100.0							
Belgium		Products for special nutritional use	2008	1		50		100.0							
Belgium		Products for special nutritional use	2010	1		50		100.0							
Belgium		Vegetable-based meals	2008	1		50		100.0							
Belgium		Snack food	2008	47		50		95.7	115.5		115.5	134			
Belgium		Snack food	2009	46		50		93.5	104		90	147			
Belgium		Snack food	2010	50		50		100.0							
Belgium	Flemish and Walloon retail stores	Conventional corn flakes	2003–2004	130	8		50.7		10	27	4		Paepens et al. (2005a)		
Belgium	Flemish and Walloon retail stores	Organic corn flakes	2003–2004	75	8		6.7		16	43	11				

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Belgium		Maize	2008	21		50		100.0					EFSA		
Belgium		Maize	2009	15		50		86.7	81		81	103			
Belgium		Maize	2010	15		50		100.0							
Belgium		Maize starch	2008	1		50		100.0							
Belgium		Maize starch	2009	2		50		100.0							
Belgium		Maize starch	2010	2		50		50.0	62		62	62			
Bulgaria		Harvested maize	2007	19	4	12	10.5		296	816			Manova & Mladenova (2009)	Co-occurrence with ZEA	Data corrected for recovery
Cyprus		Grains for human consumption	2007	1	6	20	100.0						EFSA		
Cyprus		Breakfast cereals	2006	1	6	20	100.0								
Cyprus		Fruiting vegetables	2007	10	6	20	100.0								
Czech Republic		Grains as crops	2007	27	100	100	66.7	7.4	258		265	437			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Denmark		Raisins		9	0	1	22.2		3.4	7.5			Knudsen et al. (2011)	FB_4 detected in 5 samples	
Denmark		Raisins		1	0	1	100.0								
Denmark		Raisins		2	0	1	100.0								
Denmark		Raisins		5	0	1	80.0		1.4						
Denmark		Raisins		3	0	1	66.7		4.8					FB_4 detected in 1 sample	
Denmark		Raisins		1	0	1	100.0								
Estonia		Oats	2009	1		25		100.0					EFSA		
Estonia		Barley	2009	1		25			61.2		61.2	61.2			
Estonia		Barley	2010												
Estonia		Rye feed	2009	1		25			49.8		49.8	49.8			
Estonia		Wheat	2009	3		25			104.3		112.2	139.3			
Estonia		Compound feedingstuffs	2009	4		25		25.0	176.2		182.3	242.2			
France		Grains as crops	2006	278	76	283	9.4	7.2	828		511.5	1738	EFSA		

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
France		Grains as crops	2007	208	76	297	44.7	36.1	245.1		125.5	573			
France		Grains as crops	2008	179	76	297	65.4	33.5	137		137	144			
France		Grains as crops	2009	166	76	297	56.0	40.4	547.5		479.5	979			
Germany		Grains and grain-based products	2007	4	7	25	100.0	0.0							
Germany		Grains for human consumption	2001	1		600	0.0	100.0							
Germany		Grains for human consumption	2004	5	50	60	40.0	0.0	59.8		65	74.3			
Germany		Grains for human consumption	2005	9	20	40	55.6	11.1	151.7		204	239			
Germany		Grains for human consumption	2006	42	20	40	42.9	26.2	83.7		58	137			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Grains for human consumption	2007	22	20	40	54.5	22.7	37.1		28	60			
Germany		Grains for human consumption	2008	50	35	50	52.0	42.0	167.3		108	317			
Germany		Grains for human consumption	2009	17	25	25	76.5	17.6	128.2		128.2	128.2			
Germany		Grain milling products	2000	1	20	100	0.0	100.0							
Germany		Grain milling products	2002	30	25	100	73.3	6.7	177.8		145	340			
Germany		Grain milling products	2003	151	25	50	49.0	21.9	292.6		181	690			
Germany		Grain milling products	2004	134	30	60	15.7	14.9	348.3		219	692			
Germany		Grain milling products	2005	120	30	60	39.2	32.5	135.7		23	96			
Germany		Grain milling products	2006	156	30	50	34.6	41.0	209.1		58.3	624			

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Grain milling products	2007	115	35	100	49.6	13.0	142.8		118	280			
Germany		Grain milling products	2008	258	30	100	44.2	32.9	69.1		50	127			
Germany		Grain milling products	2009	207	30	50	65.7	13.0	66.1		53	99.3			
Germany		Bread and rolls	2004	1	9	15	0.0	0.0	354		354	354			
Germany		Bread and rolls	2005	3	9	15	33.3	0.0	341		341	619			
Germany		Bread and rolls	2006	6	20	40	66.7	0.0	362.5		362.5	408			
Germany		Bread and rolls	2007	13	9	25	92.3	0.0	40		40	40			
Germany		Bread and rolls	2009	46	20	40	97.8	0.0	5.9		5.9	5.9			
Germany		Pasta (raw)	2006	20	10	20	10.0	10.0	142.9		128	184			
Germany		Pasta (raw)	2007	44	9	25	97.7	2.3							
Germany		Pasta (raw)	2009	4	9	15	75.0	0.0	122		122	122			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Breakfast cereals	2003	85	25	50	56.5	25.9	30.3		25	49.1			
Germany		Breakfast cereals	2004	28	30	60	71.4	21.4	20.1		20.1	28.1			
Germany		Breakfast cereals	2005	107	30	60	57.9	5.6	15.1		12	23.9			
Germany		Breakfast cereals	2006	178	30	100	48.3	37.1	58.5		50.9	88			
Germany		Breakfast cereals	2007	32	35	100	87.5	12.5							
Germany		Breakfast cereals	2008	58	10	25	65.5	22.4	37.1		32	76			
Germany		Breakfast cereals	2009	143	30	50	50.3	39.9	61.6		26.1	110.8			
Germany		Breakfast cereals	2010	14	25		100.0	0.0							
Germany		Fine bakery wares	2004	28	30	60	53.6	3.6	152.3		143.5	257			
Germany		Fine bakery wares	2005	64	30	60	29.7	23.4	66.2		27.6	197.5			
Germany		Fine bakery wares	2006	79	30	100	45.6	36.7	59.9		51.5	121.3			

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Fine bakery wares	2007	68	30	100	41.2	20.6	56.2		40	121			
Germany		Fine bakery wares	2008	61	16	45	52.5	13.1	56.1		57.7	79.7			
Germany		Fine bakery wares	2009	98	30	50	49.0	41.8	39.9		41.5	60.5			
Germany		Fruiting vegetables	2005	2		20	0.0	50.0	22.7		22.7	22.7			
Germany		Fruiting vegetables	2007	13	7	25	100.0	0.0							
Germany		Potatoes and potato products	2008	6	7	25	100.0	0.0							
Germany		Sausages	2007	1	7	25	0.0	0.0	32.4		32.4	32.4			
Germany		Milk and milk product imitations	2006	1	30		100.0	0.0							
Germany		Vegetable oil	2005	3	20	40	100.0	0.0							
Germany		Vegetable oil	2006	1	20	40	100.0	0.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Baking ingredients	2007	1	7	25	100.0	0.0							
Germany		Food for infants and small children	2006	1	10	20	100.0	0.0							
Germany		Infant formula, powder	2006	2	10	20	100.0	0.0							
Germany		Follow-on formula, powder	2003	1		20	0.0	100.0							
Germany		Follow-on formula, powder	2007	1	7	25	100.0	0.0							
Germany		Follow-on formula, powder	2008	1	30	100	0.0	100.0							
Germany		Follow-on formula, powder	2010	1	25		100.0	0.0							

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Cereal-based food for infants and young children	2002	44	20	40	31.8	68.2							
Germany		Cereal-based food for infants and young children	2004	9	20		100.0	0.0							
Germany		Cereal-based food for infants and young children	2005	1	40	100	100.0	0.0							
Germany		Cereal-based food for infants and young children	2006	90	30	100	44.4	53.3	48.5		48.5	54			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Cereal-based food for infants and young children	2008	11	30	100	27.3	72.7							
Germany		Cereal-based food for infants and young children	2010	12	25		100.0	0.0							
Germany		Ready-to-eat meal for infants and young children	2006	4	20	40	100.0	0.0							
Germany		Ready-to-eat meal for infants and young children	2008	4	30	100	0.0	100.0							
Germany		Products for special nutritional use	2006	1	10	20	100.0	0.0							

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Products for special nutritional use	2009	1	9	15	0.0	0.0	119		119	119			
Germany		Medicinal food[a]	2006	99	30	100	32.3	38.4	150.4		106.2	302.3			
Germany		Medicinal food[a]	2007	30	25	25	70.0	13.3	91.8		51	199			
Germany		Medicinal food[a]	2008	49	20	45	59.2	16.3	58.3		40.2	83.3			
Germany		Medicinal food[a]	2009	4	11	45	75.0	25.0							
Germany		Cereal-based dishes	2006	14	20	40	14.3	21.4	56.6		51	93			
Germany		Cereal-based dishes	2007	1	30	100	0.0	100.0							
Germany		Cereal-based dishes	2009	1	20	40	100.0	0.0							
Germany		Ready-to-eat soups	2006	2	30		100.0	0.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Snack food	2008	10	4	12	100.0	0.0							
Germany		Ices and desserts	2006	2	10	20	100.0	0.0							
Germany		Grains at harvest	2006	2	7	25	100.0	0.0							
Germany		Maize at harvest	2006	44		50		47.7	460	6710			Goertz et al. (2010)	Co-occurrence with DON, ZEA	
Germany		Maize at harvest	2007	40		50		100.0						Co-occurrence with DON, ZEA	
Hungary		Grain milling products	2004										EFSA		
Hungary		Grain milling products	2007												
Hungary		Grain milling products	2008	15	5	20	0.0	86.7	120.5		120.5	196			
Hungary		Grain milling products	2010	2	5	20	0.0	0.0	65		65	105			

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Hungary		Bread and rolls	2008	1	5	20	0.0	0.0	83		83	83			
Hungary		Breakfast cereals	2008	3	5	20	0.0	100.0							
Hungary		Breakfast cereals	2010	1	5	20	0.0	0.0	25		25	25			
Hungary		Fine bakery wares	2008												
Hungary		Vegetables and vegetable products (including fungi)	2009	1	5	20	0.0	100.0							
Hungary		Other fruit products (excluding beverages)	2009	1	5	20	0.0	100.0							
Hungary		Snack food	2008	1	5	20	0.0	0.0	99		99	99			
Hungary		Snack food	2010	1	5	20	0.0	0.0	37.9		37.9	37.9			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Hungary		Oat middlings	2008	1	5	20		100.0							
Hungary		Spelt	2008	1	5	20		100.0							
Hungary		Maize middlings	2008	1	5	20		0.0	44		44	44			
Hungary		Maize middlings	2010	1	5	20		0.0	142		142	142			
Hungary		Maize middlings	2008	2	5	20		50.0	73		73	73			
Hungary		Maize middlings	2009	1	5	20		100.0							
Hungary		Maize middlings	2010	7	5	20		71.4	47.5		47.5	67			
Italy	Perugia	Organic corn flour	2007	1		2		0.0	53	53			D'Arco et al. (2009)		
Italy		Organic corn flakes	2007	1		2		100.0							
Italy		Organic corn products	2007	7		2		14.3	21	53					
Italy		Conventional baby food	2007	9		2		100.0							

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Italy		Conventional corn flour	2007	3		2		33.3	133	187					
Italy		Conventional corn flakes	2007	10		2		100.0							
Italy		Conventional pasta	2007	14		2		85.7	4	4					
Italy		Conventional cookies	2007	11		2		81.8	5	6					
Italy		Conventional corn products	2007	26		2		80.8	20	58					
Italy		Corn flakes	2001	8		5		12.5	61.4	235			Solfrizzo, De Girolamo & Visconti (2001)		
Italy		Mixed cereals	2001	3		5		0.0	36.7	62					
Italy		Crunchy nut	2001	1		5		0.0	3						
Italy		Miel pops	2001	1		5		0.0	35						
Italy		Cruscam	2001	1		5		0.0	40						

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Italy		Cao croks	2001	1		5		0.0	10					
Italy		Sugar coated	2001	1		5		0.0	8					
Italy		Choco	2001	1		5		0.0	7					
Italy		Mixed cereals and cocoa	2001	1		5		0.0	8					
Italy		Raw cereals (maize, wheat barley, farro, rice, etc.)	March 2001 – February 2002	111	5		69.0			420	60		Cirillo et al. (2003a)	Co-occurrence with DON data available	
Italy		Bread, toast, special breads	March 2001 – February 2002	24	5		67.0			400	118			Co-occurrence with DON data available	
Italy		Durum wheat pasta	March 2001 – February 2002	17	5		0.0			790	170			Co-occurrence with DON data available	

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Italy		Breakfast cereals (corn flakes, muesli, etc.)	March 2001 – February 2002	14	5		50.0			380	65			Co-occurrence with DON data available	
Italy		Biscuits	March 2001 – February 2002	24	5		75.0			220	88			Co-occurrence with DON data available	
Italy		Baby and infant foods	March 2001 – February 2002	12	5		100.0							Co-occurrence with DON data available	
Italy		Pig liver							Traces				Gazzotti et al. (2011)		No HFB_1 or HFB_2 detected
Italy		Pig liver							Traces						No HFB_1 detected
Italy		Pig liver							Traces						No HFB_1 detected

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Italy		Pig liver							Traces						No HFB_1 detected
Italy		Pig liver							Traces						No HFB_1 detected
Italy		Pig liver							Traces						No HFB_1 detected
Italy		Pig liver													HFB_1 detected at level of 17.36 µg/kg
Italy	Apulia market	Red wine	2004–2008	45	0		80.0		0.9	2.4			Logrieco et al. (2010)		FB_4 analysed for but not detected
Italy	Apulia market	White wine	2004–2008	5	0		100.0								FB_4 analysed for but not detected
Italy	Apulia market	Rosé wine	2004–2008	1	0		100.0								FB_4 analysed for but not detected
Lithuania		Breakfast cereals	2009	1	6	21		100.0					EFSA		
Lithuania		Breakfast cereals	2010	2	6	21		100.0							

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Lithuania		Barley	2009	1	6	21		100.0					EFSA		
Lithuania		Maize	2009	4	6	21		100.0							
Lithuania		Maize	2010	4	6	21		100.0							
Lithuania		Compound feedingstuffs	2009	2	6	21		100.0							
Lithuania		Compound feedingstuffs	2010	1	6	21		100.0							
Luxembourg		Grains for human consumption	2009	4	20	60	50.0	50.0					EFSA		
Luxembourg		Grain milling products	2009	12	20	20	33.3	25.0	45.4		40.6	68.9			
Luxembourg		Pasta (raw)	2009	4	20		25.0	0.0	19.8		16.8	29.6			
Luxembourg		Breakfast cereals	2009	20	20	60	80.0	20.0							
Netherlands		Vegetable oil	2008	1	100		100.0						EFSA		
Netherlands		Vegetable oil	2005	1	100		100.0								
Netherlands		Feed	2005	7	100		100.0								
Netherlands		Feed	2006	1	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Feed	2009	2	100		100.0								
Netherlands		Cereal grains, their products and by-products	2004	1	100		100.0								
Netherlands		Cereal grains, their products and by-products	2005	3	100		100.0								
Netherlands		Cereal grains, their products and by-products	2006	3	100		100.0								
Netherlands		Cereal grains, their products and by-products	2007	1	100		100.0								
Netherlands		Cereal grains, their products and by-products	2008	7	100		100.0								
Netherlands		Cereal grains, their products and by-products	2009	3	100		100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Oats	2003	2	100		100.0								
Netherlands		Oats	2006	4	100		100.0								
Netherlands		Oats	2007	4	100		100.0								
Netherlands		Oats	2008	8	100		100.0								
Netherlands		Oats	2009	6	100		100.0								
Netherlands		Barley	2003	7	100		100.0								
Netherlands		Barley	2004	14	100		100.0								
Netherlands		Barley	2005	8	100		100.0								
Netherlands		Barley	2006	8	100		100.0								
Netherlands		Barley	2007	11	100		100.0								
Netherlands		Barley	2008	2	100		100.0								
Netherlands		Barley	2009	17	100		100.0								
Netherlands		Millet	2005	1	100		100.0								
Netherlands		Millet	2006	2	100		100.0								
Netherlands		Millet	2007	1	100		100.0								
Netherlands		Millet	2008	1	100		100.0								
Netherlands		Millet	2009	2	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Rye middlings	2006	1	100		100.0								
Netherlands		Sorghum	2006	1	100		100.0								
Netherlands		Sorghum	2007	7	100		100.0								
Netherlands		Sorghum	2008	3	100		100.0								
Netherlands		Wheat	2003	9	100		100.0								
Netherlands		Wheat	2004	23	100		100.0								
Netherlands		Wheat	2005	16	100		100.0								
Netherlands		Wheat	2006	22	100		100.0								
Netherlands		Wheat	2007	11	100		100.0								
Netherlands		Wheat	2008	6	100		100.0								
Netherlands		Wheat	2009	11	100		100.0								
Netherlands		Wheat middlings	2003	3	100		100.0								
Netherlands		Wheat middlings	2004	4	100		100.0								
Netherlands		Wheat middlings	2005	4	100		100.0								
Netherlands		Wheat middlings	2006	1	100		100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Wheat middlings	2007	3	100		100.0								
Netherlands		Wheat middlings	2009	2	100		100.0								
Netherlands		Wheat feed	2004	1	100		100.0								
Netherlands		Wheat feed	2005	1	100		100.0								
Netherlands		Wheat feed	2006	1	100		100.0								
Netherlands		Wheat feed	2007	4	100		100.0								
Netherlands		Wheat bran	2006	1	100		100.0								
Netherlands		Wheat bran	2007	1	100		100.0								
Netherlands		Wheat germ	2006	1	100		100.0								
Netherlands		Wheat gluten feed	2003	1	100		100.0								
Netherlands		Wheat gluten feed	2004	1	100		100.0								
Netherlands		Wheat gluten feed	2006	1	100		100.0								
Netherlands		Wheat starch	2005	1	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Spelt	2009	1	100		100.0								
Netherlands		Triticale	2003	4	100		100.0								
Netherlands		Triticale	2004	1	100		100.0								
Netherlands		Triticale	2005	2	100		100.0								
Netherlands		Triticale	2006	2	100		100.0								
Netherlands		Triticale	2007	3	100		100.0								
Netherlands		Triticale	2008	1	100		100.0								
Netherlands		Triticale	2009	1	100		100.0								
Netherlands		Maize	2003	29	100		82.8		4492		440	19 000			
Netherlands		Maize	2004	62	100		91.9		760		770	1300			
Netherlands		Maize	2005	67	100		89.6		310		230	620			
Netherlands		Maize	2006	57	100		100.0								
Netherlands		Maize	2007	87	100		70.1		660.4		495	1400			
Netherlands		Maize	2008	79	100		82.3		876.4		815	1500			
Netherlands		Maize	2009	38	100		100.0								
Netherlands		Maize bran	2005	1	100		100.0								
Netherlands		Maize germ expeller	2008	1	100		100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Maize gluten feed	2003	4	100		0.0		590		560	760			
Netherlands		Maize gluten feed	2004	10	100		30.0		245.7		160	740			
Netherlands		Maize gluten feed	2005	17	100		76.5		335		380	440			
Netherlands		Maize gluten feed	2006	13	100		46.2		1275.7		300	3500			
Netherlands		Maize gluten feed	2007	7	100		14.3		1926.7		1705	4000			
Netherlands		Maize gluten feed	2008	2	100		50.0		1300		1300	1300			
Netherlands		Maize gluten feed	2009	2	100		50.0		1300		1300	1300			
Netherlands		Malt culms	2007	1	100		100.0								
Netherlands		Brewers' dried grains	2005	2	100		50.0		150		150	150			
Netherlands		Brewers' dried grains	2006	4	100		50.0		385		385	490			
Netherlands		Brewers' dried grains	2009	10	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Oil seeds, oil fruits, their products and by-products	2003	8	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2004	19	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2005	21	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2006	24	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2007	19	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2008	13	100		100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Oil seeds, oil fruits, their products and by-products	2009	29	100		100.0								
Netherlands		Rape seed	2004	5	100		100.0								
Netherlands		Rape seed	2005	1	100		100.0								
Netherlands		Rape seed	2006	3	100		100.0								
Netherlands		Rape seed	2007	4	100		100.0								
Netherlands		Rape seed	2009	2	100		100.0								
Netherlands		Rape seed, expeller	2008	4	100		100.0								
Netherlands		Rape seed, expeller	2009	8	100		100.0								
Netherlands		Rape seed, extracted	2003	5	100		100.0								
Netherlands		Rape seed, extracted	2004	7	100		100.0								
Netherlands		Rape seed, extracted	2005	6	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Rape seed, extracted	2007	4	100		100.0								
Netherlands		Rape seed, extracted	2008	6	100		100.0								
Netherlands		Rape seed, extracted	2009	7	100		100.0								
Netherlands		Palm kernel expeller	2003	9	100		100.0								
Netherlands		Palm kernel expeller	2004	8	100		100.0								
Netherlands		Palm kernel expeller	2005	18	100		100.0								
Netherlands		Palm kernel expeller	2006	10	100		100.0								
Netherlands		Palm kernel expeller	2007	12	100		100.0								
Netherlands		Palm kernel expeller	2008	12	100		100.0								
Netherlands		Palm kernel expeller	2009	6	100		100.0								
Netherlands		Palm kernel, extracted	2005	1	100		100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Palm kernel, extracted	2008	1	100		100.0								
Netherlands		Soya (bean), toasted	2003	3	100		100.0								
Netherlands		Soya (bean), toasted	2004	3	100		100.0								
Netherlands		Soya (bean), toasted	2006	1	100		100.0								
Netherlands		Soya (bean), toasted	2008	2	100		100.0								
Netherlands		Soya (bean), extracted, toasted	2003	14	100		100.0								
Netherlands		Soya (bean), extracted, toasted	2004	33	100		100.0								
Netherlands		Soya (bean), extracted, toasted	2005	51	100		100.0								
Netherlands		Soya (bean), extracted, toasted	2006	54	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Soya (bean), extracted, toasted	2007	39	100		97.4		230		230	230			
Netherlands		Soya (bean), extracted, toasted	2008	43	100		95.3		185		185	240			
Netherlands		Soya (bean), extracted, toasted	2009	61	100		100.0								
Netherlands		Niger seed expeller	2009	1	100		100.0								
Netherlands		Sunflower seed	2003	2	100		100.0								
Netherlands		Sunflower seed	2004	3	100		100.0								
Netherlands		Sunflower seed	2005	4	100		100.0								
Netherlands		Sunflower seed	2006	4	100		100.0								
Netherlands		Sunflower seed	2007	1	100		100.0								
Netherlands		Sunflower seed	2009	4	100		100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Sunflower seed, extracted	2003	3	100		100.0								
Netherlands		Sunflower seed, extracted	2004	7	100		100.0								
Netherlands		Sunflower seed, extracted	2005	4	100		100.0								
Netherlands		Sunflower seed, extracted	2006	14	100		100.0								
Netherlands		Sunflower seed, extracted	2007	10	100		100.0								
Netherlands		Sunflower seed, extracted	2008	5	100		100.0								
Netherlands		Sunflower seed, extracted	2009	9	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Sunflower seed, partially decorticated, extracted	2008	1	100		100.0								
Netherlands		Linseed	2003	1	100		100.0								
Netherlands		Linseed	2004	1	100		100.0								
Netherlands		Linseed	2005	1	100		100.0								
Netherlands		Linseed	2006	1	100		100.0								
Netherlands		Linseed	2007	2	100		100.0								
Netherlands		Linseed expeller	2003	1	100		100.0								
Netherlands		Linseed expeller	2007	1	100		100.0								
Netherlands		Linseed expeller	2008	2	100		100.0								
Netherlands		Cocoa husks	2003	2	100		100.0								
Netherlands		Sweet lupins	2003	1	100		100.0								
Netherlands		Sweet lupins	2008	1	100		100.0								
Netherlands		Peas	2003	1	100		100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Peas	2004	4	100		100.0								
Netherlands		Peas	2005	4	100		100.0								
Netherlands		Peas	2006	3	100		100.0								
Netherlands		Peas	2008	2	100		100.0								
Netherlands		Tubers, roots, their products and by-products	2004	9	100		100.0								
Netherlands		Tubers, roots, their products and by-products	2005	1	100		100.0								
Netherlands		Tubers, roots, their products and by-products	2006	2	100		100.0								
Netherlands		Tubers, roots, their products and by-products	2007	4	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Tubers, roots, their products and by-products	2008	5	100		100.0								
Netherlands		(Sugar) beet pulp	2003	7	100		100.0								
Netherlands		(Sugar) beet pulp	2004	11	100		100.0								
Netherlands		(Sugar) beet pulp	2005	19	100		100.0								
Netherlands		(Sugar) beet pulp	2006	11	100		100.0								
Netherlands		(Sugar) beet pulp	2007	1	100		100.0								
Netherlands		(Sugar) beet pulp	2008	1	100		100.0								
Netherlands		Sweet potato	2006	1	100		100.0								
Netherlands		Potato pulp	2003	1	100		100.0								
Netherlands		Potato pulp	2005	1	100		100.0								
Netherlands		Potato pulp	2006	2	100		100.0								
Netherlands		Potato starch	2003	1	100		100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Potato protein	2005	1	100		100.0								
Netherlands		Potato protein	2006	1	100		100.0								
Netherlands		Other seeds and fruits, their products and by-products	2003	1	100		100.0								
Netherlands		Other seeds and fruits, their products and by-products	2004	2	100		100.0								
Netherlands		Other seeds and fruits, their products and by-products	2006	1	100		100.0								
Netherlands		Carob pods	2008	1	100		100.0								
Netherlands		Citrus pulp	2003	9	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Citrus pulp	2004	22	100		95.5		370		370	370			
Netherlands		Citrus pulp	2005	15	100		100.0								
Netherlands		Citrus pulp	2006	1	100		100.0								
Netherlands		Citrus pulp	2007	2	100		100.0								
Netherlands		Citrus pulp	2008	6	100		100.0								
Netherlands		Citrus pulp	2009	5	100		100.0								
Netherlands		Forages and roughage	2008	1	100		100.0								
Netherlands		Forages and roughage	2003	127	100		79.5		251.2		195	440			
Netherlands		Forages and roughage	2004	166	100		94.6		286.7		170	1200			
Netherlands		Forages and roughage	2005	168	100		99.4		140		140	140			
Netherlands		Forages and roughage	2006	171	100		90.1		157.1		140	200			
Netherlands		Forages and roughage	2007	76	100		90.8		211.4		150	500			
Netherlands		Forages and roughage	2008	97	100		82.5		206.5		180	310			

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Forages and roughage	2009	76	100		96.1		363.3		240	680			
Netherlands		Lucerne meal	2003	1	100		100.0								
Netherlands		Lucerne meal	2005	2	100		100.0								
Netherlands		Lucerne meal	2006	7	100		100.0								
Netherlands		Lucerne meal	2007	14	100		100.0								
Netherlands		Lucerne meal	2008	2	100		100.0								
Netherlands		Clover meal	2006	1	100		100.0								
Netherlands		Grass meal	2003	2	100		100.0								
Netherlands		Grass meal	2004	3	100		100.0								
Netherlands		Grass meal	2005	12	100		100.0								
Netherlands		Grass meal	2006	3	100		100.0								
Netherlands		Grass meal	2007	19	100		100.0								
Netherlands		Grass meal	2008	20	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Grass meal	2009	2	100		100.0								
Netherlands		Other plants, their products and by-products	2008	2	100		100.0								
Netherlands		Milk products	2005	4	100		75.0		120		120	120			
Netherlands		Milk products	2006	1	100		100.0								
Netherlands		Whey powder	2005	1	100		100.0								
Netherlands		Poultry meal	2003	13	100		100.0								
Netherlands		Poultry meal	2004	50	100		80.0		195		155	345			
Netherlands		Poultry meal	2005	36	100		86.1		672		280	1800			
Netherlands		Poultry meal	2008	2	100		0.0		240		240	250			
Netherlands		Poultry meal	2009	2	100		100.0								
Netherlands		Minerals	2006	1	100		100.0								
Netherlands		Minerals	2008	1	100		100.0								
Netherlands		Bakery and pasta products and by-products	2006	1	100		100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Bakery and pasta products and by-products	2003	1	100		100.0								
Netherlands		Bakery and pasta products and by-products	2004	5	100		100.0								
Netherlands		Bakery and pasta products and by-products	2005	10	100		100.0								
Netherlands		Bakery and pasta products and by-products	2006	14	100		100.0								
Netherlands		Bakery and pasta products and by-products	2007	1	100		100.0								
Netherlands		Bakery and pasta products and by-products	2009	1	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Confectionery products and by-products	2004	1	100		100.0								
Netherlands		Compound feedingstuffs	2004	8	100		100.0								
Netherlands		Compound feedingstuffs	2005	6	100		66.7		260		260	270			
Netherlands		Compound feedingstuffs	2006	2	100		100.0								
Netherlands		Compound feedingstuffs	2008	1	100		0.0		320		320	320			
Netherlands		Compound feedingstuffs	2009	6	100		100.0								
Portugal		Yellow maize	2005	9	15		33.0		99	272			Silva et al. (2007)		
Portugal		White maize	2005	2	15		0.0		275	437					
Portugal		Maize flour	2005	3	15		33.0		173	457					
Portugal		Maize semolina	2005	3	15		100.0								
Portugal		Maize starch	2005	3	15		100.0								
Portugal		Sweet maize	2005	11	15		100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Portugal		Cornflakes	2005	16	15		100.0								
Portugal		Mixed cereals	2005	4	15		100.0								
Portugal		Maize snacks	2005	16	15		93.8		5.6	90					
Portugal	Coimbra	Maize bread (broa)	2005	30	15		16.7		77	207	70		Lino et al. (2007)		
Portugal	Local retail markets	Cornmeal		41	20		29.3		176.9	450			Martins et al. (2008)		
Portugal	Local retail markets	Sweet corn (popcorn)		49	20		100.0								
Portugal	Local retail markets	Corn flakes		15	20		100.0								
Portugal		Mixed feed for laying hens		52	100		100.0						Martins, Guerra & Bernardo (2006)	Co-occurrence with ZEA and DON	
Slovakia		Grains for human consumption	2008	2	30	100		100.0					EFSA		

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Slovakia		Grains for human consumption	2009	9	30	100		77.8	45.5		45.5	48.5			
Slovakia		Grain milling products	2008	3	30	100		33.3	179		179	268			
Slovakia		Grain milling products	2009	17	30	100		94.1	232.6		232.6	232.6			
Slovakia		Bread and rolls	2008	2	30	100		100.0							
Slovakia		Bread and rolls	2009	11	30	100		90.9	255		255	255			
Slovakia		Breakfast cereals	2009	2	15	41		100.0							
Slovakia		Fine bakery wares	2009	3	15	41		100.0							
Slovakia		Fruiting vegetables	2008	5	15	41		0.0	392.9		316	928.1			
Slovakia		Fruiting vegetables	2009	2	30	100		100.0							
Slovakia		Snack food	2008	1	30	100		100.0							
Slovakia		Snack food	2009	12	30	100		91.7	301		301	301			

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Slovakia		Grains as crops	2008	1	15	41		0.0	77.3		77.3	77.3			
Slovakia		Grains as crops	2009	3	30	100		100.0							
Spain		Grains for human consumption	2008	7		100	0.0	100.0							
Spain		Grains for human consumption	2009	6		100	0.0	100.0							
Spain		Grains for human consumption	2010	7		100	0.0	100.0							
Spain		Grain milling products	2009	8		60	0.0	87.5	270		270	270			
Spain		Grain milling products	2010	5		200	0.0	100.0							
Spain		Bread and rolls	2009	12		60	0.0	100.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Spain		Bread and rolls	2010	5		60	0.0	100.0							
Spain		Pasta (raw)	2008	4		100	0.0	100.0							
Spain		Pasta (raw)	2009	4		100	0.0	100.0							
Spain		Pasta (raw)	2010	4		100	0.0	100.0							
Spain		Breakfast cereals	2008	1		100	0.0	100.0							
Spain		Breakfast cereals	2009	10		60	0.0	100.0							
Spain		Breakfast cereals	2010	3		200	0.0	100.0							
Spain		Fine bakery wares	2009	6		60	0.0	100.0							
Spain		Fine bakery wares	2010	5		60	0.0	100.0							
Spain		Cereal-based food for infants and young children	2008	6		100	0.0	100.0							

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Spain		Cereal-based food for infants and young children	2009	16		100	0.0	100.0							
Spain		Ready-to-eat meal for infants and young children	2009	1		60	0.0	100.0							
Spain		Snack food	2009	10		60	0.0	90.0	93		93	93			
Spain		Snack food	2010	3		200	0.0	66.7	240		240	240			
Spain	Valencia	Corn flour	2006	9	12	35	44.4		336	644			Silva et al. (2009)		
Spain		Sweet corn	2006	6	12	35	100.0								
Spain		Corn snacks	2006	9	12	35	100.0								
Spain		Corn flakes	2006	11	12	35	100.0								
Spain		Bread and rolls	2006	3	12	35	100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (μg/kg)	LOQ (μg/kg)	% < LOD	% < LOQ	Mean (μg/kg)	Maximum (μg/kg)	Median (μg/kg)	90th percentile (μg/kg)			
Spain	Valencia	Organic baby food	2007	5		2		40.0	80	229			D'Arco et al. (2009)		
Spain		Organic corn flakes	2007	1		2		100.0							
Spain		Organic cookies	2007	1		2		0.0	17	17					
Spain		Organic corn products	2007	4		2		25.0	5	7.5					
Spain		Conventional baby food	2007	48		2		93.8	3	3					
Spain		Conventional corn flour	2007	7		2		28.6	5	9					
Spain		Conventional corn flakes	2007	11		2		81.8	4	9					
Spain		Conventional cookies	2007	5		2		60.0	19	29					
Spain		Conventional corn products	2007	22		2		86.4	9	15					

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Spain	Aragón	Conventional corn grain	2001–2003	30		25		90.0	21.9**	120			Ariño, Estopañan & González-Cabo (2007)		
Spain	Aragón	Organic corn grain	2001–2003	30		25		93.3	18.8**	153					
Europe		Other feed ingredients	2006	3	25	80	66.7		25	25			Submitted to JECFA		
Europe		Other feed ingredients	2006	3	25	80	33.3		384.5	722					
Europe		Other feed ingredients	2006	3	25	80	100.0								
Europe		Maize (feed)	2006	4	25	80	25.0		110.5*	328					
Europe		Maize (feed)	2006	1	25	80	100.0								
Europe		Soya bean	2006	2	25	80	100.0								
Europe		Maize (feed)	2007	5	25	80	20.0		720.4*	2726					
Europe		Other feed ingredients	2007	5	25	80	0.0		260.4	737					
Europe		Other feed ingredients	2007	4	25	80	0.0		169.5	218					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Europe		Other feed ingredients	2008	27	25	80	25.9		658.6	5094					
Europe		Other feed ingredients	2008	4	25	80	50.0		216	231					
Europe		Finished feed	2008	3	25	80	66.7		54	54					
Europe		Finished feed	2008	6	25	80	33.3		378.2*	1152					
Europe		Maize (feed)	2009	6	25	80	0.0		653.8	1658					
Europe		Maize (feed)	2009	6	25	80	0.0		403	716					
Europe		Maize (feed)	2009	8	25	80	75.0		71.4*	287					
Europe		Cereal	2009	13	25	80	84.6		91*	622					
Europe		Other feed ingredients	2009	6	25	80	100.0								
Europe		Finished feed	2009	16	25	80	93.8		217	217					
Europe		Finished feed	2009	3	25	80	33.3		176.5	196					
Europe		Finished feed	2009	4	25	80	100.0								

Table A1-2 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_2 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Europe		Finished feed	2009	2	25	80	100.0								
Europe		Finished feed	2009	2	25	80	0.0		793.5	1112					
Europe		Breeder layer mash	2009	2	25	80	50.0		194	194					

AFB_1, aflatoxin B_1; DON, deoxynivalenol; EFSA, European Food Safety Authority; ELISA, enzyme-linked immunosorbent assay; HPLC, high-performance liquid chromatography; LOD, limit of detection; LOQ, limit of quantification; MS/MS, tandem mass spectrometry; ND, not detected; OTA, ochratoxin A; SAGPYA, Secretaría de Agricultura, Ganadería, Pesca y Alimentos de la Nación, Argentina; ZEA, zearalenone

* = Mean of all samples where non-detects are assigned a value of zero.

** = Mean or median calculated using one half the LOQ or LOD for results lower than the LOQ or LOD.

[a] Specially formulated and intended for the dietary management of a disease that has distinctive nutritional needs that cannot be met by normal diet alone; intended to be used under medical supervision.

Table A1-3. Occurrence of fumonisin B_3 in food (unshaded) and feed (shaded) commodities and their ingredients

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Americas															
Argentina	Buenos Aires, Entre Rios, Cordoba provinces	Corn puff	2007	20	24		70.0		22	286			Federico et al. (2010)		
Argentina		Grain maize	2006	187	25	50	18.7		58.4	566.5	50.1	196.4	Sampling under Resolution No. 1075/94 SAGPYA		
Argentina	Cordoba Province	Corn flour	2005	23	100		100.0						Lerda et al. (2005)		
Argentina		Rice grain	2005	29	100		100.0								
Brazil	Sao Paulo State	Infant cereal type C	2004	16	20		37.5		43	111	46		de Castro et al. (2004)		With corn flour
Brazil		Cornmeal	2004	89	20		0.0		154	549	122				
Brazil		Infant cereal type A-B-D	2004	46	20		100.0								A-B corn starch, D minor corn flour
Brazil		Corn starch	2004	33	20		100.0								
Brazil		Instant corn-based baby food	2004	12	20		41.7		29	58	24				

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil	Santa Catarina West	Corn grain	1990–2000	39	18		0.0		360				Van der Westhuizen et al. (2003)		
Brazil	Santa Catarina North	Corn grain	1990–2000	17	18		0.0		300						
Brazil	Santa Catarina South	Corn grain	1990–2000	20	18		10.0		100						
Brazil	Santa Catarina South	Corn grain intended for feeds	1990–2000	14	18		0.0		110						
Guatemala		Corn at shops	2009	16	7		0.0		390	1830	140	1090	Riley, Torres & Palencia (2006)		
Guatemala		Incaparina (mixture of corn and cottonseed flour + vitamins)	2000	3	16		100.0						Trucksess et al. (2002)	Yes	Corrected by recovery
USA		Incaparina (mixture of corn and cottonseed flour + vitamins)	1998	5					60	200	50			Yes	
Asia															
Islamic Republic of Iran	Fars	Corn	2000	15	10		86.7		7	64			Ghiasian et al. (2006)		Occurrence of 3-*epi*-FB_3 was calculated

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Islamic Republic of Iran	Khuzestan	Corn	2000	14	10		85.7		5	48					
Islamic Republic of Iran	Kermanshah	Corn	2000	12	10		91.7		3	33					
Islamic Republic of Iran	Mazandaran Province	Corn	2000	11	10		0.0		596	900					
Japan		Raw corn	2004–2007	41		10		100.0					Aoyama et al. (2010)		
Japan		Frozen or canned corn	2004–2007	127		10		100.0							
Japan		Popcorn grain	2004–2007	57		2		29.8	8.7	64					
Japan		Corn grits	2004–2007	46		2		15.2	19.3	358					
Japan		Corn flakes	2004–2007	81		10		100.0							
Japan		Corn soups	2004–2007	88		10		100.0							

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Corn starch	2004–2007	22		2		100.0						
Japan		Corn snacks	2004–2007	50		2		28.0	14	281				
Japan		Beer	2004–2007	30		2		100.0						
Japan		Buckwheat flour	2004–2007	15		10		100.0						
Japan		Buckwheat dried noodles	2004–2007	50		2		100.0						
Japan		Flattened barley	2004–2007	40		10		100.0						
Japan		Soya beans	2004–2007	82		2		100.0						
Japan		Polished rice	2004–2007	31		4		100.0						
Japan		Millet	2004–2007	30		2		100.0						
Japan		Asparagus	2004–2007	20		2		100.0						

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Corn	2007–2008	28	0.6	2		0.0	157.8	610	120	410	Submitted to JECFA		
Japan		Corn grits	2004–2009	53	0.6	2		9.4	27.4	358	10.8	59.5			
Japan		Raw corn	2004–2009	51	1	10		100.0	<LOD	2.1	<LOD	<LOD			
Japan		Popcorn	2004–2009	69	1	2		36.2	7.1	64	3.9	17.7			
Japan		Sweet corn	2004–2009	126	5	10		100.0	<LOD	4	<LOD	<LOD			
Japan		Corn flakes	2004–2009	101	5	10		99.0	0.2	14.6	<LOD	<LOD			
Japan		Corn soup (liquid)	2004–2009	63	5	10		100.0	<LOD	<LOD	<LOD	<LOD			
Japan		Corn soup (powder)	2004–2009	46	5	10		100.0	<LOD	<LOD	<LOD	<LOD			
Japan		Corn starch	2004–2009	35	1	2		91.4	0.3	7.1	<LOD	<LOD			
Japan		Corn snack	2004–2009	90	1	2		25.6	11.9	281	5.7	14.7			

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Raw corn	2004–2005	18		10		100.0					Sugita-Konishi et al. (2006)		
Japan		Canned or frozen corn	2004–2005	51		10		100.0							
Japan		Buckwheat dried noodles	2004–2005	30		2		100.0							
Japan		Popcorn	2004–2005	15		2		26.6	12.8	64					
Japan		Corn flakes	2004–2005	30		10		100.0							
Japan		Corn soup	2004–2005	29		10		100.0							
Japan		Corn grits	2004–2005	10		2		0.0	13.1	18					
Japan		Flattened barley	2004–2005	20		10		100.0							
Japan		Corn feed	2004–2005	48	0.6	2	0.0		47	230			Aoyama & Ishikuro (2007)		
Japan		Corn gluten meal (feed)	2004–2005	2	0.6	2	0.0		30	39					
Japan		Hominy feed	2004–2005	3	0.6	2	0.0		71	110					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Grain sorghum (feed)	2004–2005	11	0.6	2	9.0		11	46					
Japan		Barley (feed)	2004–2005	14	0.6	2	50.0		2.2	13					
Japan		Rye (feed)	2004–2005	3	0.6	2	0.0		5.3	12					
Japan		Wheat feed	2004–2005	2	0.6	2	50.0		<LOQ	3.1					
Japan		Dehulled rice (feed)	2004–2005	3	0.6	2	100.0		<LOD	<LOD					
Japan		Soya bean meal (feed)	2004–2005	5	0.6	2	80.0		<LOQ	2.8					
Japan		Cotton seed (feed)	2004–2005	2	0.6	2	100.0		<LOD	<LOD					
Japan		Alfalfa feed	2004–2005	2	0.6	2	100.0		<LOD	<LOD					
Japan		Beet pulp (feed)	2004–2005	5	0.6	2	60.0		4.7	20					
Japan		Formula (feed)	2004–2005	9	0.6	2	0.0		39	87					
Turkey	Germencincik	Dried figs	2004							92	25	Karbancioglu-Güler & Heperkan (2009)		
Turkey	Incirliova	Dried figs	2004							90	25			
Turkey	Ortaklar	Dried figs	2004							90	25			

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Turkey	Selcuk	Dried figs	2004							78	25			
Turkey	Soke	Dried figs	2004							47	25			
Turkey	Torbali	Dried figs	2004							47	25			
Africa														
Ghana	Accra	Maize from markets	1996	14	50		57.1		114.5	226			Kpodo, Sorensen & Jakobsen (2000)		
Ghana	Five agroecological zones	Maize from processing sites	1999	75	10		49.3		73.2	267			Kpodo (2001)	Co-occurrence with aflatoxins	
Ghana	Accra	Maize from markets	2000	15	10		20.0		86.9	263					
Ghana	Five agroecological zones	Processed maize (kenkey)	1999	75	10		85.3		29.8	66				Co-occurrence with aflatoxins	
Ghana	Accra	Processed maize (kenkey)	2000	15	10		80.0		37.7	52					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Nigeria	Niger State	Rice (field samples)	2008	10	15		100.0						Makun et al. (2011)	Co-occurrence with aflatoxins, OTA, ZEA, DON	
Nigeria	Niger State	Rice (stored samples)	2008	6	15		100.0							Co-occurrence with aflatoxins, OTA, ZEA, DON	
Nigeria	Niger State	Rice (market samples)	2008	5	15		100.0							Co-occurrence with aflatoxins, OTA, ZEA, DON	
Europe															
Belgium		Grain milling products	2008	10		50		60.0	111.3		88	198	EFSA		
Belgium		Grain milling products	2009	21		50		90.5	72.5		72.5	94			
Belgium		Grain milling products	2010	32		50		87.5	66.5		60	91			

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Belgium		Bread and rolls	2008	7		50		100.0							
Belgium		Bread and rolls	2009	12		50		75.0	89.3		81	114			
Belgium		Bread and rolls	2010	6		50		100.0							
Belgium		Pasta (raw)	2009	11		50		100.0							
Belgium		Pasta (raw)	2010	10		50		100.0							
Belgium		Breakfast cereals	2008	12		50		100.0							
Belgium		Breakfast cereals	2009	10		50		100.0							
Belgium		Breakfast cereals	2010	12		50		100.0							
Belgium		Fine bakery wares	2009	1		50		0.0	61		61	61			
Belgium		Tree nuts	2009	1		50		100.0							
Belgium		Food for infants and small children	2010	4		50		100.0							
Belgium		Cereal-based food for infants and young children	2008	10		50		100.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Belgium		Cereal-based food for infants and young children	2009	9		50		100.0							
Belgium		Cereal-based food for infants and young children	2010	6		50		100.0							
Belgium		Ready-to-eat meal for infants and young children	2009	1		50		100.0							
Belgium		Products for special nutritional use	2008	1		50		100.0							
Belgium		Products for special nutritional use	2010	1		50		100.0							
Belgium		Vegetable-based meals	2008	1		50		100.0							
Belgium		Snack food	2008	47		50		95.7	55.5		55.5	56			
Belgium		Snack food	2009	46		50		93.5	55.3		53	60			
Belgium		Snack food	2010	50		50		100.0							

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Belgium	Flemish and Walloon retail stores	Conventional corn flakes	2003–2004	130	12.5		50.8		18	50	19		Paepens et al. (2005a)		
Belgium	Flemish and Walloon retail stores	Organic corn flakes	2003–2004	75	12.5		6.7		26	90	19				
Belgium		Maize	2008	21		50		100.0					EFSA		
Belgium		Maize	2009	15		50		93.3	194		194	194			
Belgium		Maize	2010	15		50		100.0							
Belgium		Maize starch	2008	1		50		100.0							
Belgium		Maize starch	2009	2		50		100.0							
Germany		Breakfast cereals	2007	28	30	100	92.9	7.1					EFSA		
Germany		Breakfast cereals	2008	51	9	25	66.7	31.4	58.1		58.1	58.1			
Germany		Breakfast cereals	2009	38	20	40	86.8	5.3	37.2		36	50			
Germany		Breakfast cereals	2010	14	25		100.0	0.0							
Germany		Fine bakery wares	2004	18	30	60	27.8	11.1	58.7		64	83			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Fine bakery wares	2005	47	30	60	42.6	34.0	55		50	83.3			
Germany		Fine bakery wares	2006	70	30	100	50.0	42.9	36.7		27.9	79			
Germany		Fine bakery wares	2007	66	30	100	45.5	33.3	35.5		28.3	63.8			
Germany		Fine bakery wares	2008	32	9	25	43.8	21.9	39.2		42	46			
Germany		Fine bakery wares	2009	59	25	50	62.7	35.6	27.5		27.5	27.5			
Germany		Fruiting vegetables	2005					0.0							
Germany		Fruiting vegetables	2007	13	7	25	100.0								
Germany		Potatoes and potato products	2008	6	7	25	100.0	0.0							
Germany		Sausages	2007	1	7	25	0.0	0.0	29.9		29.9	29.9			
Germany		Vegetable oil	2005	3	20	40	100.0								
Germany		Vegetable oil	2006	1	20	40	100.0	0.0							
Germany		Baking ingredients	2007	1	7	25	100.0	0.0							

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Food for infants and small children	2007	1	7	25	100.0								
Germany		Food for infants and small children	2008	1	30	100	0.0	100.0							
Germany		Food for infants and small children	2010	1	25		100.0	0.0							
Germany		Food for infants and small children	2006	49	30	100	51.0	49.0							
Germany		Food for infants and small children	2008	8	30	100	0.0	100.0							
Germany		Food for infants and small children	2010	12	25		100.0	0.0							
Germany		Ready-to-eat meal for infants and young children	2006	4	20	40	100.0	0.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Ready-to-eat meal for infants and young children	2008	4	30	100	0.0	100.0							
Germany		Ready-to-eat meal for infants and young children	2009	1	9	15	0.0	0.0	39		39	39			
Germany		Medicinal food[a]	2006	27	30	100	3.7	81.5	148.5		154.5	172			
Germany		Medicinal food[a]	2007	30	25	25	80.0	13.3	83		83	101			
Germany		Medicinal food[a]	2008	23	20	40	69.6	21.7	29.5		29.5	33.1			
Germany		Medicinal food[a]	2009	3	9	25	66.7	33.3							
Germany		Cereal-based dishes	2006	13	20	40	38.5	53.8	41		41	41			
Germany		Cereal-based dishes	2007	1	30	100	0.0	100.0							
Germany		Cereal-based dishes	2009	1	20	40	100.0	0.0							
Germany		Snack food	2008	2	2.8	9.1	100.0	0.0							
Germany		Grains at harvest	2006	2	7	25	50.0	0.0	27.5		27.5	27.5			

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Italy	Perugia	Organic corn flour	2007	1		5		0.0	24	24			D'Arco et al. (2009)		
Italy		Organic corn flakes	2007	1		5		100.0							
Italy		Organic corn products	2007	7		5		14.0	15	28					
Italy		Conventional baby food	2007	9		5		100.0							
Italy		Conventional corn flour	2007	3		5		33.3	63	70					
Italy		Conventional corn flakes	2007	10		5									
Italy		Conventional pasta	2007	14		5		100.0							
Italy		Conventional cookies	2007	11		5		100.0							
Italy		Conventional corn products	2007	26		5		80.8	16	30					
Netherlands		Vegetable oil	2008	1	100		100.0						EFSA		
Netherlands		Vegetable oil	2005												
Netherlands		Feed	2005	7	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Feed	2006	1	100		100.0								
Netherlands		Feed	2009	2	100		100.0								
Netherlands		Cereal grains, their products and by-products	2004												
Netherlands		Cereal grains, their products and by-products	2005	3	100		100.0								
Netherlands		Cereal grains, their products and by-products	2006	3	100		100.0								
Netherlands		Cereal grains, their products and by-products	2007	1	100		100.0								
Netherlands		Cereal grains, their products and by-products	2008	7	100		100.0								
Netherlands		Cereal grains, their products and by-products	2009	3	100		100.0								
Netherlands		Oats	2006	4	100		100.0								
Netherlands		Oats	2007	4	100		100.0								
Netherlands		Oats	2008	8	100		100.0								

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Oats	2009	6	100		100.0								
Netherlands		Barley	2005	4	100		100.0								
Netherlands		Barley	2006	8	100		100.0								
Netherlands		Barley	2007	11	100		100.0								
Netherlands		Barley	2008	2	100		100.0								
Netherlands		Barley	2009	17	100		100.0								
Netherlands		Millet	2006	2	100		100.0								
Netherlands		Millet	2007	1	100		100.0								
Netherlands		Millet	2008	1	100		100.0								
Netherlands		Millet	2009	2	100		100.0								
Netherlands		Rye middlings	2006	1	100		100.0								
Netherlands		Sorghum	2006	1	100		100.0								
Netherlands		Sorghum	2007	7	100		100.0								
Netherlands		Sorghum	2008	3	100		100.0								
Netherlands		Wheat	2005	6	100		100.0								
Netherlands		Wheat	2006	22	100		100.0								
Netherlands		Wheat	2007	11	100		100.0								
Netherlands		Wheat	2008	6	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Wheat	2009	11	100		100.0								
Netherlands		Wheat middlings	2005	2	100		100.0								
Netherlands		Wheat middlings	2006	1	100		100.0								
Netherlands		Wheat middlings	2007	3	100		100.0								
Netherlands		Wheat middlings	2009	2	100		100.0								
Netherlands		Wheat feed	2006	1	100		100.0								
Netherlands		Wheat feed	2007	4	100		100.0								
Netherlands		Wheat bran	2006	1	100		100.0								
Netherlands		Wheat bran	2007	1	100		100.0								
Netherlands		Wheat germ	2006	1	100		100.0								
Netherlands		Wheat gluten feed	2006	1	100		100.0								
Netherlands		Spelt	2009	1	100		100.0								
Netherlands		Triticale	2005	1	100		100.0								
Netherlands		Triticale	2006	2	100		100.0								
Netherlands		Triticale	2007	3	100		100.0								

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Triticale	2008	1	100		100.0								
Netherlands		Triticale	2009	1	100		100.0								
Netherlands		Maize	2005	27	100		92.6		125		125	130			
Netherlands		Maize	2006	57	100		100.0								
Netherlands		Maize	2007	87	100		82.8		324.7		200	710			
Netherlands		Maize	2008	79	100		87.3		314		285	575			
Netherlands		Maize	2009	38	100		100.0								
Netherlands		Maize bran	2005	1	100		100.0								
Netherlands		Maize germ expeller	2008	1	100		100.0								
Netherlands		Maize gluten feed	2005	11	100		72.7		133.3		130	150			
Netherlands		Maize gluten feed	2006	13	100		76.9		156.7		140	200			
Netherlands		Maize gluten feed	2007	7	100		14.3		483.3		445	900			
Netherlands		Maize gluten feed	2008	2	100		50.0		360		360	360			
Netherlands		Maize gluten feed	2009	2	100		50.0		530		530	530			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Malt culms	2007	1	100		100.0								
Netherlands		Brewers' dried grains	2006	4	100		50.0		210		210	250			
Netherlands		Brewers' dried grains	2009	10	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2005	8	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2006	24	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2007	19	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2008	13	100		100.0								
Netherlands		Oil seeds, oil fruits, their products and by-products	2009	29	100		100.0								

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Rape seed	2005	1	100		100.0								
Netherlands		Rape seed	2006	3	100		100.0								
Netherlands		Rape seed	2007	4	100		100.0								
Netherlands		Rape seed	2009	2	100		100.0								
Netherlands		Rape seed, expeller	2008	4	100		100.0								
Netherlands		Rape seed, expeller	2009	8	100		100.0								
Netherlands		Rape seed, extracted	2005	2	100		100.0								
Netherlands		Rape seed, extracted	2007	4	100		100.0								
Netherlands		Rape seed, extracted	2008	6	100		100.0								
Netherlands		Rape seed, extracted	2009	7	100		100.0								
Netherlands		Palm kernel expeller	2005	12	100		100.0								
Netherlands		Palm kernel expeller	2006	10	100		100.0								
Netherlands		Palm kernel expeller	2007	12	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Palm kernel expeller	2008	12	100		100.0								
Netherlands		Palm kernel expeller	2009	6	100		100.0								
Netherlands		Palm kernel, extracted	2008	1	100		100.0								
Netherlands		Soya (bean), toasted	2006	1	100		100.0								
Netherlands		Soya (bean), toasted	2008	2	100		100.0								
Netherlands		Soya (bean), extracted, toasted	2005	34	100		100.0								
Netherlands		Soya (bean), extracted, toasted	2006	54	100		100.0								
Netherlands		Soya (bean), extracted, toasted	2007	39	100		100.0								
Netherlands		Soya (bean), extracted, toasted	2008	43	100		100.0								

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Soya (bean), extracted, toasted	2009	61	100		100.0								
Netherlands		Niger seed expeller	2009	1	100		100.0								
Netherlands		Sunflower seed	2006	4	100		100.0								
Netherlands		Sunflower seed	2007	1	100		100.0								
Netherlands		Sunflower seed	2009	4	100		100.0								
Netherlands		Sunflower seed, extracted	2005	2	100		100.0								
Netherlands		Sunflower seed, extracted	2006	14	100		100.0								
Netherlands		Sunflower seed, extracted	2007	10	100		100.0								
Netherlands		Sunflower seed, extracted	2008	5	100		100.0								
Netherlands		Sunflower seed, extracted	2009	9	100		100.0								
Netherlands		Sunflower seed, partially decorticated, extracted	2008	1	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Linseed	2005	1	100		100.0								
Netherlands		Linseed	2006	1	100		100.0								
Netherlands		Linseed	2007	2	100		100.0								
Netherlands		Linseed expeller	2007	1	100		100.0								
Netherlands		Linseed expeller	2008	2	100		100.0								
Netherlands		Sweet lupins	2008	1	100		100.0								
Netherlands		Peas	2005	1	100		100.0								
Netherlands		Peas	2006	3	100		100.0								
Netherlands		Peas	2008	2	100		100.0								
Netherlands		Tubers, roots, their products and by-products	2005	1	100		100.0								
Netherlands		Tubers, roots, their products and by-products	2006	2	100		100.0								
Netherlands		Tubers, roots, their products and by-products	2007	4	100		100.0								

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Tubers, roots, their products and by-products	2008	5	100		100.0								
Netherlands		(Sugar) beet pulp	2005	15	100		100.0								
Netherlands		(Sugar) beet pulp	2006	11	100		100.0								
Netherlands		(Sugar) beet pulp	2007	1	100		100.0								
Netherlands		(Sugar) beet pulp	2008	1	100		100.0								
Netherlands		Sweet potato	2006	1	100		100.0								
Netherlands		Potato pulp	2006	2	100		100.0								
Netherlands		Potato protein	2006	1	100		100.0								
Netherlands		Other seeds and fruits, their products and by-products	2006	1	100		100.0								
Netherlands		Carob pods	2008	1	100		100.0								
Netherlands		Citrus pulp	2005	9	100		100.0								
Netherlands		Citrus pulp	2006	1	100		100.0								
Netherlands		Citrus pulp	2007	2	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Citrus pulp	2008	6	100		100.0								
Netherlands		Citrus pulp	2009	5	100		100.0								
Netherlands		Forages and roughage	2008	1	100		100.0								
Netherlands		Forages and roughage	2005	84	100		100.0								
Netherlands		Forages and roughage	2006	171	100		99.4		170		170	170			
Netherlands		Forages and roughage	2007	76	100		97.4		130		130	130			
Netherlands		Forages and roughage	2008	97	100		96.9		126.7		110	160			
Netherlands		Forages and roughage	2009	76	100		98.7		220		220	220			
Netherlands		Lucerne meal	2005	1	100		100.0								
Netherlands		Lucerne meal	2006	7	100		100.0								
Netherlands		Lucerne meal	2007	14	100		100.0								
Netherlands		Lucerne meal	2008	2	100		100.0								
Netherlands		Clover meal	2006	1	100		100.0								
Netherlands		Grass meal	2005	3	100		100.0								

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Grass meal	2006	3	100		100.0								
Netherlands		Grass meal	2007	19	100		100.0								
Netherlands		Grass meal	2008	20	100		100.0								
Netherlands		Grass meal	2009	2	100		100.0								
Netherlands		Other plants, their products and by-products	2008	2	100		100.0								
Netherlands		Milk products	2005	1	100		100.0								
Netherlands		Milk products	2006	1	100		100.0								
Netherlands		Milk products	2005	14	100		92.9		610		610	610			
Netherlands		Milk products	2008	2	100		100.0								
Netherlands		Milk products	2009	2	100		100.0								
Netherlands		Minerals	2006	1	100		100.0								
Netherlands		Minerals	2008	1	100		100.0								
Netherlands		Bakery and pasta products and by-products	2006	1	100		100.0								
Netherlands		Bakery and pasta products and by-products	2005	7	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Bakery and pasta products and by-products	2006	14	100		100.0								
Netherlands		Bakery and pasta products and by-products	2007	1	100		100.0								
Netherlands		Bakery and pasta products and by-products	2009	1	100		100.0								
Netherlands		Bakery and pasta products and by-products	2005	3	100		100.0								
Netherlands		Bakery and pasta products and by-products	2006	2	100		100.0								
Netherlands		Bakery and pasta products and by-products	2008	1	100		100.0								
Netherlands		Bakery and pasta products and by-products	2009	6	100		100.0								
Spain	Valencia	Organic baby food	2007	5		5	40.0		55	105			D'Arco et al. (2009)		
Spain		Organic corn flakes	2007	1		5	0.0								

Table A1-3 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_3 occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Spain		Organic cookies	2007	1		5	0.0		11	11					
Spain		Organic corn products	2007	4		5	100.0								
Spain		Conventional baby food	2007	48		5		100.0							
Spain		Conventional corn flour	2007	7		5		85.7	4	4					
Spain		Conventional corn flakes	2007	11		5		90.9	4	4					
Spain		Conventional cookies	2007	5		5		80.0	16	16					
Spain		Conventional corn products	2007	22		5		95.4	11	11					

AFB_1, aflatoxin B_1; DON, deoxynivalenol; EFSA, European Food Safety Authority; LOD, limit of detection; LOQ, limit of quantification; ND, not detected; OTA, ochratoxin A; SAGPYA, Secretaría de Agricultura, Ganadería, Pesca y Alimentos de la Nación, Argentina; ZEA, zearalenone

* = Mean of all samples where non-detects are assigned a value of zero.

** = Mean or median calculated using one half the LOQ or LOD for results lower than the LOQ or LOD.

[a] Specially formulated and intended for the dietary management of a disease that has distinctive nutritional needs that cannot be met by normal diet alone; intended to be used under medical supervision.

Table A1-4. Occurrence of total fumonisins in food (unshaded) and feed (shaded) commodities and their ingredients

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Americas															
Argentina	Buenos Aires, Entre Rios, Cordoba provinces	Corn puff	2007	20	61		5.0		323	1649	278		Federico et al. (2010)		
Argentina		Grain maize	2005	100	10		2.0		632.8	15 779	104.5	948.2	Sampling under Resolution No. 1075/94 SAGPYA		$FB_T = FB_1 + FB_2$
Argentina		Grain maize	2006	187	75	150	4.3		812.1	4741.8	492.7	1809.4			$FB_T = FB_1 + FB_2 + FB_3$
Argentina		Grain maize	2007	176	139.5	394.2	17.6		712.1	3038	519	1427.2	Submitted to JECFA		$FB_T = FB_1 + FB_2$
Argentina	Entre Rios Province	Corn grain	2003	14	46	128	14.6		10 200	34 700	5800		Broggi et al. (2007)	Yes	
Argentina		Corn grain	2004	17	46	128	29.4		4700	16 100	2400				
Argentina	Cordoba, Buenos Aires, Santa Fe provinces	Harvested maize grain	2007	163	16	48	2.4		3079.1	20 397	1632		Pacin et al. (2009)		$FB_T = FB_1 + FB_2$
Argentina		Stored maize grain (120–226 days)	2007	163	16	48	1.8		4238.5	31 108	2495.1				Silo bag

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Argentina	Cordoba Province	Corn flour	2005	23	300		75.0		543.5	2600	<LOD	1600	Lerda et al. (2005)		
Argentina		Rice grain	2005	29	300		89.7		89.7	900	<LOD	160			
Brazil	Sao Paulo State	Infant cereal type C	2004	16	60*		0.0		664	1753	111		de Castro et al. (2004)		With corn flour
Brazil		Cornmeal	2004	89	60*		0.0		2242	8039	549				
Brazil		Infant cereal type A-B-D	2004	46	60*		100.0								A-B corn starch, D minor corn flour
Brazil		Corn starch	2004	33	60*		100.0								
Brazil		Instant corn-based baby food	2004	12	60*		0.0		437	1096	58				
Brazil	Sao Paulo State	Cornmeal	2000	30	50		0.0		6170	19 230			Bittencourt et al. (2005)		
Brazil		Corn flour	2000	30	50		0.0		2740	8880					
Brazil	Paraná State	Freshly harvested corn	1996	36	93		0.0		9900	22 600			Ono et al. (2002)		
Brazil		Stored corn	1996	36	93		0.0		9900	23 700					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil	Paraná State	Maize freshly after harvesting	2003	100			0.0		2480	15 320	1510		Ono et al. (2008b)		$FB_T = FB_1 + FB_2$
Brazil		Maize industrial delivery posts	2003	200			0.0		2430	15 900	1720				
Brazil		Maize before drying	2003	90			0.0		3170	18 780	2040				
Brazil	Northern Paraná State	Corn (reception)	2003	300	62.8*		0.0		2240	15 900			Ono et al. (2008a)		$FB_T = FB_1 + FB_2$
Brazil		Corn (pre-drying)	2003	135	62.8*		0.0		2870	18 780					
Brazil		Corn (reception)	2004	300	62.8*		0.0		1460	18 160					
Brazil		Corn (pre-drying)	2004	135	62.8*		0.0		1520	11 210					
Brazil	Northern Paraná State	Maize freshly after harveshing	2004	100			0.0		1020	4780			Da Silva et al. (2008)		
Brazil		Maize industrial delivery posts	2004	100			0.0		1110	4090					
Brazil		Maize before drying	2004	45			0.0		1570	11 210					
Brazil	Federal District	Cornmeal I (fubà)	2003–2005	62		20		0.0	1680	6170			Caldas & Silva (2007)		$FB_T = FB_1 + FB_2$

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil		Cornmeal II (creme de milho)	2003–2005	11		20		0.0	2040	3440					
Brazil		Precooked flour I (beiju)	2003–2005	21		20		0.0	653	2050					
Brazil		Precooked flour II (milharina)	2003–2005	21		20		0.0	1090	2380					
Brazil		Snacks	2003–2005	20		20		15.0	178	555					
Brazil		Corn flakes	2003–2005	20		20		60.0	127	906					
Brazil		Popcorn	2003–2005	24		20		8.3	664	2100					
Brazil		Sweet corn, on the cob	2003–2005	6		20		100.0							
Brazil		Sweet corn, frozen	2003–2005	8		20		62.5	354	671					
Brazil		Sweet corn, canned	2003–2005	15		20		80.0	190	1440					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil		Corn gluten	2009	73	100		28.8		4437.2	20 800	2760	9616	Submitted to JECFA		
Brazil		Corn bran	2009	10	100		33.3		3403.3	14 880	2070	8456			
Brazil		Degermed corn	2009	5	100		20.0		568	960	640	928			
Brazil		Corn flakes		20		40		60.0	125	906	<LOQ	507.7			
Brazil		Corn flour (fine)		11		40		0.0	2045.7	3440	1116.5	3289			
Brazil		Precooked corn flour		42		40		0.0	872.5	2380	1116.5	1453			
Brazil		Corn flour		61		40		0.0	1678.4	6174	1369	2801.7			
Brazil		Popcorn		24		40		8.3	664.5	2097	614.5	1232.6			
Brazil		Corn snack		20		40		5.0	176.2	555	138.5	402.1			
Brazil		Sweet corn, frozen		8		40		50.0	70.9	201	28	170.2			
Brazil		Sweet corn, canned		15		20		80.0	190	1440					
Brazil	Paraná State	Corn kernels	1995	24	130		0.0		6327	20 380	5460	11 420	Ono et al. (2006)		
Brazil	Santa Catarina State	Commercial corn flour	2001	25	90		8.0		6357.7	21 823	4365	15 387	Scaff & Scussel (2004)		$FB_T = FB_1 + FB_2$

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil		Home-processed corn flour	2001	22	90		9.0		3811.6	19 222	1311.5	10 699			
Brazil		Canjica	2001	12	90		16.6		732.3	2237	621	1534.1			
Brazil		Corn flakes	2001	11	90		0.0		1215.7	5856	807	1419			
Brazil		Popcorn	2001	12	90		8.3		2872.2	9773	2556.5	8038			
Brazil	Paraná State	Freshly harvested corn (reception)	2003	90	62.8		0.0		2540	15 320			Moreno et al. (2009)	Yes	$FB_T = FB_1 + FB_2$
Brazil		Freshly harvested corn (pre-drying)	2003	60	62.8		0.0		3120	18 780					
Brazil		Freshly harvested corn (reception)	2004	90	62.8		1.1		1310	18 160					
Brazil		Freshly harvested corn (pre-drying)	2004	60	62.8		5.0		1360	6280					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Brazil		Corn, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2010	431	100		12.0		2515	53 700			Rodrigues & Naehrer (2011a)	Yes	
Brazil		Corn, barley, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2011	103	100		33.0		1781.8	31 050			Rodrigues & Naehrer (2011b)	Yes	
Brazil	Paraná State	Corn kernels intended for feeds	1995	24	130		0.0		185 192	336 380	183 095	286 090	Ono et al. (2006)		
Brazil	Bahia State	Grain barley for feed	2007	50	262		27.5		908.5				Batatinha et al. (2007); Simas et al. (2007)	Yes	$FB_T = FB_1 + FB_2$

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Canada		Corn-based breakfast cereals	1999–2001	34	4	10	11.7		114	1980			Roscoe et al. (2008)	Yes	$FB_T = FB_1 + FB_2$
Canada		Multigrain breakfast cereals	1999–2001	24	4	10	54.2		12	88					
Canada		Oat-based breakfast cereals	1999–2001	19	4	10	73.7		6	57					
Canada		Rice-based breakfast cereals	1999–2001	29	4	10	93.1		0.3	5					
Canada		Wheat-based breakfast cereals	1999–2001	29	4	10	82.7		3	51					
Canada		Buckwheat cereals	1999–2001	1	4	10	0.0		5	5					
Canada		Oat-based cereals	1997–1999	5		20		100.0					Lombaert et al. (2003)	Yes	$FB_T = FB_1 + FB_2$
Canada		Barley-based cereals	1997–1999	10		20		100.0							LOD calculated as $LOD_{FB1} + LOD_{FB2}$
Canada		Soya-based cereals	1997–1999	24		20		25.0	33	130					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Canada		Rice-based cereals	1997–1999	25		20		96.0	<LOQ	10					
Canada		Multigrain cereals	1997–1999	69		20		68.1	14	120					
Canada		Teething biscuits	1997–1999	2		20		50.0	10	20					
Canada		Soya formulas	1997–1999	1		20		100.0							
Canada		Creamed corn	1997–1999	6		20		100.0							
Guatemala		Incaparina (mixture of corn and cottonseed flour + vitamins)	2000	3	48		0.0		800	1700	500		Trucksess et al. (2002)	Yes	Corrected by recovery
Guatemala		Maize collected from commercial vendors	2005	236	10		11.4		3550	43 080					
USA		Incaparina (mixture of corn and cottonseed flour + vitamins)	1998	5					840	2200	300			Yes	

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
USA		Corn, whole	2004	64		100		53.1	910	5840			Submitted to JECFA		$FB_T = FB_1 + FB_2$
USA		Corn, canned	2004	7		100		0.0	880	1630					
USA		Corn flour	2004	49		100		67.3	730	4290					
USA		Corn grits	2004	36		100		75.0	530	1200					
USA		Cornmeal	2004	123		100		65.0	600	3390					
USA		Popcorn	2004	23		100		100.0							
USA		Corn products	2004	15		100		73.3	1880	7980					
USA		Corn, whole	2005	39		100		41.0	310	2440					$FB_T = FB_1 + FB_2$
USA		Corn, canned	2005	7		100		42.9	280	410					
USA		Corn flour	2005	38		100		44.7	380	2680					
USA		Corn grits	2005	18		100		72.2	140	348					
USA		Cornmeal	2005	96		100		53.1	510	4040					
USA		Popcorn	2005	17		100		94.1	30	510					
USA		Corn products	2005	25		100		48.0	1140	1540					
USA		Corn, whole	2006	41		100		36.6	820	4680					$FB_T = FB_1 + FB_2$

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
USA		Corn flour	2006	32		100		46.3	830	3570					
USA		Corn grits	2006	4		100		7.3	77.5	310					
USA		Cornmeal	2006	62		100		48.8	420	3690					
USA		Popcorn (unpopped)	2006	20		100		43.9	1100	760					
USA		Corn products (various)	2006	23		100		29.3	420	2100					
USA		Breakfast cereal	2006	7		100		4.9	630	1990					
USA		Corn, whole	2007	19		100		21.1	1100	5530					
USA		Corn flour	2007	34		100		29.4	520	2050					
USA		Corn grits	2007	7		100		28.5	320	420					
USA		Cornmeal	2007	36		100		19.4	560	2900					
USA		Popcorn (unpopped)	2007	12		100		75.0	240	580					
USA		Corn products (various)	2007	10		100		40.0	960	3830					
USA		Corn, whole	2008	24		100		12.5	480	1540					
USA		Corn, canned		4		100		0.0	860	2570					
USA		Corn flour	2008	13		100		15.4	430	1630					
USA		Corn grits	2008	8		100		25.0	480	1290					

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
USA		Cornmeal	2008	37		100		29.7	580	2900					
USA		Popcorn (unpopped)	2008	30		100		80.0	540	1630					
USA		Corn products (various)	2008	4		100		50.0	600	671					
USA		Breakfast cereal	2008	5		100		40.0	600	2300					
USA	Central Illinois	Popcorn	2005	5	10		20.0		244	500	210	476	Dowd & Johnson (2010)	Yes	
USA	Central Illinois	Popcorn	2006	8	10		0.0		785	1680	745	1400		Yes	
USA	Central Illinois	Popcorn	2007	7	10		0.0		218.4	710	58	668		Yes	
USA	Central Illinois	Popcorn	2008	7	10		0.0		312.8	820	170	610		Yes	
USA		Feed (DDGS)	2007–2008	20	100		5.0		1880	8600	700	4730	Zhang et al. (2009)	Yes	
USA		Feed (DDGS)	2006–2007	69	100		0.0		2327	5880	2010	4348		Yes	
USA		Feed (DDGS)	2006–2007	16	100		0.0		1471.3	2770	1465	2225		Yes	
USA		Feed (DDGS)	2008	77	100	200		2.6	2687	7200	2300	5160		Yes	

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
USA		Feed (DDGS, from China, Province of Taiwan)	2006	7	100		0.0		2300	2900				Yes	Ethanol plants
USA		Feed (DDGS, from China, Province of Taiwan)	2006	22	100		0.0		1550	2400				Yes	Port containers
USA		Feed (DDGS, from China, Province of Taiwan)	2007	24	100		0.0		1200	2400				Yes	Port containers
North and South America (USA, Argentina, Brazil)		Corn, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2006–2007	48	100		37.0		409.2	7910			Rodrigues (2008)	Yes	

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
North America (USA, Canada)		Corn, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2010	243	100		51.0		695	22 900			Rodrigues & Naehrer (2011a)	Yes	
North America (USA, Canada)		Corn, barley, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2011	10	100		40.0		700.2	2088			Rodrigues & Naehrer (2011b)	Yes	
Asia															
China	Shandong Province	Asparagus	2004	30	15	80			157.7	714	97.5	395.8	C. Liu et al. (2005)		$FB_T = FB_1 + FB_2$
China	Province of Taiwan	Corn	2002	20	100		65.0		26.5	130	0	81	F.-M. Liu et al. (2005)		$FB_T = FB_1 + FB_2$

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
China	Province of Taiwan	Fresh corn	2002	5	100		80.0		30	150	0	90			
China	Province of Taiwan	Corn snack	2002	15	100		86.6		14	160	0	0			
China	Province of Taiwan	Corn flakes	2002	10	100		100.0								
China	Province of Taiwan	Corn starch	2002	5	100		100.0								
China	Province of Taiwan	Canned corn	2002	5	100		100.0								
China	Province of Taiwan	Corn raw material	2002	16	100		93.7		5.6	90	0	0			
China	Tibet Autonomous Region, Shigatze Prefecture	Barley	1997	25	200		44.0		450	1100			Haubruge et al. (2003)	Yes	
Indonesia		Industrially produced foods	2001	24	8.7		41.7		50.1	104.6	49.1		Nuryono et al. (2002)		
Iraq	Nineveh	Poultry feeds	2005–2007	45	50		49.9		127	350	10	200	Shareef (2010)	Yes	

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Islamic Republic of Iran	Fars	Corn	2000	15	10				215	1228			Ghiasian et al. (2006)		Occurrence of 3-*epi*-FB_3 was calculated
Islamic Republic of Iran	Khuzestan	Corn	2000	14	10				174	1138					$FB_T = FB_1 + FB_2 + FB_3 +$ 3-*epi*-FB_3
Islamic Republic of Iran	Kermanshah	Corn	2000	12	10				71	494					
Islamic Republic of Iran	Mazandaran Province	Corn	2000	11	10				10 674	15 447					
Islamic Republic of Iran		Maize	2001–2002	4	20		25.0		243	415			Ghiasian et al. (2009)		$FB_T = FB_1 + FB_2$
Islamic Republic of Iran		Maize	2001–2002	4	20		25.0		268	412					
Islamic Republic of Iran		Maize	2001–2002	6	20		34.0		254	512					
Islamic Republic of Iran		Maize	2001–2002	3	20		34.0		116	235					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Islamic Republic of Iran		Maize	2001–2002	10	20		40.0		114	325					
Islamic Republic of Iran		Maize	2001–2002	6	20		50.0		100	356					
Japan		Corn feed	2004–2005	48						3000			Aoyama & Ishikuro (2007)		
Japan		Corn gluten meal (feed)	2004–2005	2						340					
Japan		Hominy feed	2004–2005	3						1700					
Japan		Grain sorghum (feed)	2004–2005	11						480					
Japan		Barley (feed)	2004–2005	14						63					
Japan		Rye (feed)	2004–2005	3						39					
Japan		Wheat feed	2004–2005	2						12					
Japan		Dehulled rice (feed)	2004–2005	3						<LOD					

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Japan		Soya bean meal (feed)	2004–2005	5						9.2					
Japan		Cotton seed (feed)	2004–2005	2						<LOD					
Japan		Alfalfa feed	2004–2005	2						<LOD					
Japan		Beet pulp (feed)	2004–2005	5						76					
Japan		Formula (feed)	2004–2005	9						1100					
Republic of Korea		Corn kernels	2008	78	15	50		17.9	850	9980			Submitted to JECFA		
Republic of Korea		Corn flour	2008	21	15	50		28.6	190	790					
Republic of Korea		Frozen corn	2008	19	15	50		94.7	10	50					
Republic of Korea		Canned corn	2008	21	15	50		57.1	50	280					
Republic of Korea		Popcorn	2008	12	15	50		91.7	60	710					
Republic of Korea		Corn snack	2008	83	15	50		59.0	130	1090					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Republic of Korea		Frozen corn (boiled)	2008	14	45		57.1		20	90			Chung et al. (2008)		
Republic of Korea		Kernel corn	2008	39	45		7.7		1560	13 000					
Republic of Korea		Corn flour	2008	10	45		0.0		290	1060					
Republic of Korea		Corn grits	2008	8	45		12.5		370	870					
Republic of Korea		Corn snack	2008	20	45		10.0		420	1360					
Republic of Korea		Corn cereal	2008	3	45		0.0		20	20					
Republic of Korea		Popped corn	2008	12	45		100.0								
Republic of Korea		Canned corn	2008	14	45		100.0								
Republic of Korea		Roasted corn	2008	11	45		0.0		90	260					
Turkey		Maize for feed	2002–2003	19	25				88 240	356 800			Oruc, Cengiz & Kalkanli (2006)	Yes	
Turkey		Maize for feed	2002–2003	7	25				74 150	262 500					

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Asia	North Asia (China, including Province of Taiwan, Republic of Korea and Japan)	Corn, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2006–2007	397	100		34.0		1238.8	21 484			Rodrigues (2008)	Yes	
Asia	South-east Asia (Malaysia, Philippines, Thailand, Viet Nam, Indonesia)	Corn, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2006–2007	269	100		46.0		541.1	10 766				Yes	
Asia	South Asia (India, Pakistan, Bangladesh)	Corn, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2006–2007	20	100		45.0		182.3	1455				Yes	

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Asia	North Asia (China, including Province of Taiwan, Republic of Korea and Japan)	Corn, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2010	732	100		56.0		780	13 862			Rodrigues & Naehrer (2011a)	Yes	
Asia	South-east Asia (Malaysia, Philippines, Thailand, Viet Nam, Indonesia)	Corn, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2010	369	100		47.0		493	6196					
Asia	South Asia (India, Pakistan, Bangladesh)	Corn, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2010	89	100		46.0		323	1852					

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Asia	North, South-east and South Asia	Corn, barley, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2011	267	100		55.0		289.4	10 979			Rodrigues & Naehrer (2011b)	Yes	Mean calculated from positive samples
Oceania															
Oceania	Australia and New Zealand	Corn, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2006–2007	49	100		90.0		150.1	2795			Rodrigues (2008)	Yes	
Oceania	Australia and New Zealand	Corn, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2010	149	100		91.0		87	3229			Rodrigues & Naehrer (2011a)	Yes	

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Africa															
Burkina Faso	Kenedougou Province	Stored maize	1999	26	10		0.0		1170	3120	810		Nikièma et al. (2004)		
Burkina Faso	Kenedougou Province	Maize at harvest	1999	26	10		0.0		140	450	90				
Burkina Faso	Banfora	Maize from markets	1999	18	10				3390	16 040	2050				
Burkina Faso	Basi Satiri	Maize from markets	1999	1	10				1500						
Burkina Faso	Dande	Maize from markets	1999	1	10				2910						
Burkina Faso	N'Dorola	Maize from markets	1999	13	10				1800	7910	720				
Burkina Faso	Kanyan	Maize from markets	1999	3	10				1230	2080	1200				
Burkina Faso	Kourouma	Maize from markets	1999	18	10				1750	3890	1430				
Burkina Faso		Maize from markets	1999	3	10				3470	4760	3170				
Burkina Faso		Maize from markets	1999	7	10				6840	9690	6660				

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Ghana	Five agroecological zones	Maize from processing sites	1999	75	30		9.3		412	2534			Kpodo (2001)	Co-occurrence with aflatoxins	
Ghana	Five agroecological zones	Processed maize (kenkey)	1999	75	30		26.7		228.1	1035				Co-occurrence with aflatoxins	
South Africa	North-western Bizana	Home-grown maize	2001	7	5				1020	3975			Van der Westhuizen et al. (2010a)		
South Africa	North-western Bizana	Home-grown maize	2002	9	5				485	1430					
South Africa	North-western Bizana	Home-grown maize	2003	9	5				90	500					
South Africa	North-western Bizana	Commercial maize meal	2001–2003	9	5				530	1580					
South Africa	South-eastern Centane	Home-grown maize	2001	3	5				1140	1955					

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
South Africa	South-eastern Centane	Home-grown maize	2002	9	5				540	1980					
South Africa	South-eastern Centane	Home-grown maize	2003	3	5				565	1205					
South Africa	South-eastern Centane	Commercial maize meal	2001–2003	11	5				235	900					
United Republic of Tanzania	Kilimanjaro	Stored maize	2005	120	100		48.0			11 048	363		Kimanya et al. (2008)	Co-occurrence with aflatoxins	Results corrected for recovery
United Republic of Tanzania	Kilimanjaro	Freshly harvested maize	2006	67	60		6.0		1718	22 817	185		Kimanya et al. (2009)		Results corrected for recovery
United Republic of Tanzania	Kilimanjaro	Stored maize	2006	55	60		72.7		485	1758	139				Results corrected for recovery
Zimbabwe		Maize (feed)	2006	1		250		0.0	1520	1520			Submitted to JECFA		
Zimbabwe		Maize (feed)	2006												$FB_T = FB_1 + FB_2$
Zimbabwe		Maize (feed)	2007	2		250		0.0	3235	3460					

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Zimbabwe		Other feed ingredients	2008	5	50		0.0		682.8	1599					$FB_T = FB_1 + FB_2$
Zimbabwe		Other feed ingredients	2008	46	50		4.3		704.9*	1285					$FB_T = FB_1 + FB_2$
Zimbabwe		Finished feed	2009	11	50		36.4		422.5*	1181					$FB_T = FB_1 + FB_2$
Zimbabwe		Finished feed	2009	111	50		27.0		693.1*	2674					$FB_T = FB_1 + FB_2$
Zimbabwe		Silage	2009	6	50		0.0		990	1402					$FB_T = FB_1 + FB_2$
Zimbabwe		Silage	2009	2	50		100.0								$FB_T = FB_1 + FB_2$
Zimbabwe		Other feed ingredients	2009	38	50		86.8		89.7*	2035					$FB_T = FB_1 + FB_2$
Zimbabwe		Cottonseed (feed)	2009	3	50		100.0								
Zimbabwe		Maize (feed)	2009	49	50		14.3		1298*	4398					$FB_T = FB_1 + FB_2$
Zimbabwe		Maize (feed)	2009	2	50		50.0		692	692					$FB_T = FB_1 + FB_2$

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Zimbabwe		Cereals (feed)	2009	25	50		80.0		551.2*	10 485					$FB_T = FB_1 + FB_2$
Zimbabwe		Soya bean (feed)	2009	2	50		100.0								
Zimbabwe		Soya bean meal	2009	3	50		100.0								
Europe															
Austria		Grains for human consumption	2008	1	20	100	0.0	100.0					EFSA		
Austria		Grains for human consumption	2009	3	20	100	100.0	0.0							
Austria		Grain milling products	2007	68	20	100	61.8	19.1	255		172	586			
Austria		Grain milling products	2008	12	20	100	50.0	16.7	407.2		128.4	1262			
Austria		Grain milling products	2009	10	20	100	100.0	0.0							
Austria		Bread and rolls	2008	3	20	100	100.0	0.0							
Austria		Pasta (raw)	2008	9	20	100	33.3	44.4	406.5		406.5	426			
Austria		Breakfast cereals	2007	35	20	100	94.3	5.7							

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Austria		Breakfast cereals	2008	6	20	100	16.7	33.3	217		175	302			
Austria		Breakfast cereals	2009	5	20	100	60.0	40.0							
Austria		Fine bakery wares	2008	2	20	100	50.0	0.0	106		106	106			
Austria		Vegetables and vegetable products (including fungi)	2007	1	20	100	100.0	0.0							
Austria		Dried fruits	2007	1	20	100	100.0	0.0							
Austria		Beer and beer-like beverages	2008	1	20	100	100.0	0.0							
Austria		Cereal-based food for infants	2007	1	20	100	100.0	0.0							
Austria		Cereal-based food for infants	2008	1	20	100	100.0	0.0							
Austria		Cereal-based food for infants	2008	2	20	100	50.0	50.0							
Austria		Other foods	2008	1	20	100	100.0	0.0							

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Bulgaria		Harvested maize	2007	19	27.3	90.8	5.3		1150	4050			Manova & Mladenova (2009)	Co-occurrence with ZEA	Data corrected for recovery
Croatia	Brodsko-Posavska County	Maize	2007	12	200		75.0		7630	20 700			Segvic Klaric et al. (2009)	Co-occurrence with aflatoxins, OTA and ZEA	
Croatia	Brodsko-Posavska County	Wheat	2007	6	200		100.0							Co-occurrence with aflatoxins, OTA and ZEA	
Croatia	Brodsko-Posavska County	Barley	2007	4	200		100.0							Co-occurrence with aflatoxins, OTA and ZEA	
Croatia	Brodsko-Posavska County	Oat	2007	2	200		100.0							Co-occurrence with aflatoxins, OTA and ZEA	

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Croatia	Brodsko-Posavska County	Feed	2007	13	200		46.2		2300	5000			Segvic Klaric et al. (2009)	Co-occurrence with aflatoxins, OTA and ZEA	
Czech Republic		Grains as crops	2007	42	50	50	50.0		275.1		190	521	EFSA		
Czech Republic		Grains as crops	2008	32	50	50	87.5		139.4		96.7	305.4			
Czech Republic		Grains as crops	2009	28	50	50	82.1		1864.5		131	8804.4			
Czech Republic		Grains as crops	2010	22	50	50	90.9		357.3		357.3	374.7			
France		Grains as crops	2007	428	250	200	65.2	13.3	826.5		256	2189			
France		Grains as crops	2008	721	250	200	54.5	27.9	511.3		249	1239			
France		Grains as crops	2009	363	250	200	28.1	28.1	1187.5		436	2943			
France		Grains for human consumption	2007	61	222	100	96.7	1.6	394		394	394			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
France		Grains for human consumption	2008	34	222	100	97.1	2.9							
France		Grain milling products	2007	9	222	100	66.7	33.3							
France		Grain milling products	2008	10	222	100	50.0	40.0	878		878	878			
France		Grain milling products	2009	9	222	222	22.2	44.4	1333.3		1121	1933			
France		Bread and rolls	2008	3			0.0	0.0	83.3		100	100			
France		Breakfast cereals	2009	2		50	0.0	100.0							
France		Breakfast cereals											Molinié et al. (2005)	Co-occurrence with OTA	
France		Cereal grains, their products and by-products	2007	11	222	100	36.4		3852.1		1420	15 584	EFSA		
France		Cereal grains, their products and by-products	2008	7	222		57.1		490.3		334	803			
France		Oats	2008	1		100	0.0	100.0							

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
France		Wheat middlings	2007	2	222		50.0		2745		2745	2745			
France		Wheat middlings	2008	4	222		100.0								
France		Triticale	2007	28	222		82.1		366.4		272	753			
France		Triticale	2008	32	222		93.7		519		519	519			
France		Maize gluten feed	2008	5	222	20	0.0		190.6		104	357			
France		Maize gluten feed	2009	5	222	100	0.0		286.7		345	426			
France		Maize gluten	2008	6	222	100	50.0		377		377	505			
France		Maize gluten	2009	2	222	200	0.0		1195		1195	1195			
Germany		Grains for human consumption	2001	1	680	880	0.0	100.0					EFSA		
Germany		Grains for human consumption	2006	14			0.0	0.0	135.4		74.5	455.3			
Germany		Grain milling products	2000	1	20	100	0.0	0.0	290		290	290			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Grain milling products	2002	11	20	100	36.4	0.0	820		650	1630			
Germany		Grain milling products	2003	24	25	50	54.2	4.2	1770.1		1489.5	4681.5			
Germany		Grain milling products	2004	1			0.0	0.0	65		65	65			
Germany		Grain milling products	2005	19	9		63.2	0.0	114.8		50.3	348.3			
Germany		Grain milling products	2006	59			0.0	0.0	172.6		44.3	639			
Germany		Grain milling products	2007	1	10	30	0.0	0.0	664		664	664			
Germany		Grain milling products	2008	43	10	30	0.0	11.6	236.8		131.7	514.5			
Germany		Grain milling products	2009	16			0.0	0.0	95.6		57.8	238			
Germany		Pasta (raw)	2006	1	9		100.0	0.0							
Germany		Breakfast cereals	2003	11	25	50	9.1	36.4	172.8		82	548			
Germany		Breakfast cereals	2004	6	30	60	16.7	0.0	31.5		30	44			

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Breakfast cereals	2005	2			0.0	0.0	64		64	88			
Germany		Breakfast cereals	2006	55	10	30	1.8	14.5	92.5		40.4	324			
Germany		Breakfast cereals	2009	9			0.0	0.0	64.7		16.5	453			
Germany		Breakfast cereals	2005	2			0.0	0.0	425.8		425.8	687			
Germany		Breakfast cereals	2006	40	10	30	20.0	32.5	125.2		35	448.8			
Germany		Breakfast cereals	2009	10			0.0	0.0	130.3		120	273			
Germany		Breakfast cereals	2008	1	10	30	0.0	100.0							
Germany		Cereal-based food for infants and young children	2002	34	200	1000	61.8	11.8	152.9		33	1110			
Germany		Cereal-based food for infants and young children	2006	38	10	30	0.0	63.2	9.6		7	25			

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Germany		Cereal-based food for infants and young children	2008	8	10	30	0.0	87.5	90.5		90.5	90.5			
Germany		Ready-to-eat meal for infants and young children	2006	1			0.0	0.0	7		7	7			
Germany		Ready-to-eat meal for infants and young children	2008	4	10	30	0.0	100.0							
Germany	Lower Saxony	Popcorn maize	2009	12		25		33.3	176	577	<LOQ		Reinhold & Reinhardt (2011)		$FB_T = FB_1 + FB_2$
Germany	Lower Saxony	Maize snacks	2009	30		25		53.3	48	80	<LOQ				$FB_T = FB_1 + FB_2$
Germany	Lower Saxony	Maize flour and polenta	2009	23		25		47.8	117	340	26				$FB_T = FB_1 + FB_2$
Germany	Lower Saxony	Tortilla chips	2009	10		25		50.0	163	260	30				$FB_T = FB_1 + FB_2$
Germany		Maize meal and semolina	2004	37	5		8.0		823	6617			Engelhardt, Barthel & Sparrer (2006)		$FB_T = FB_1 + FB_2$

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Greece/ Cyprus		Feed	January 2005 – August 2009	2		25		0.0	1371*	2274	1371		Griessler et al. (2010)	Co-occurrence with DON, ZEA, OTA, aflatoxins	$FB_T = FB_1 + FB_2$
Hungary		Grains as crops	2008	69	222	666	88.4	5.8	827.5		750	1120	EFSA		
Hungary		Grains as crops	2009	20	222	666	90.0	5.0	1540		1540	1540			
Hungary		Grains as crops	2010	13	222	666	69.2	15.4	1678		1678	2430			
Hungary		Compound feedingstuffs	2008	56	222	666	51.8	21.4	1150.9		1080	1540	EFSA		
Hungary		Compound feedingstuffs	2009	22	222	666	59.1	18.2	879.5		750	1360			
Hungary		Compound feedingstuffs	2010	15	222	666	33.3	26.7	1681.5		1675	3340			
Italy	Southern Italy	Cornmeal	2008–2009	21	9	15		0.0	310	1334			Magro et al. (2011)		Values corrected for recovery; $FB_T = FB_1 + FB_2$
Italy	Southern Italy	Maize flour	2008–2009	8	9	15		0.0	914	2309					Values corrected for recovery; $FB_T = FB_1 + FB_2$

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (μg/kg)	LOQ (μg/kg)	% < LOD	% < LOQ	Mean (μg/kg)	Maximum (μg/kg)	Median (μg/kg)	90th percentile (μg/kg)			
Italy	Southern Italy	Maize	2008–2009	7	9	15		43.0	401	1342					Values corrected for recovery; $FB_T = FB_1 + FB_2$
Italy	Southern Italy	Corn flakes	2008–2009	35	9	15		71.0	89	178					Values corrected for recovery; $FB_T = FB_1 + FB_2$
Italy	Southern Italy	Snacks	2008–2009	16	9	15		25.0	110	561					Values corrected for recovery; $FB_T = FB_1 + FB_2$
Italy	Southern Italy	Gluten-free foods	2008–2009	13	9	15		46.0	170	618					Values corrected for recovery; $FB_T = FB_1 + FB_2$
Italy		Feed	January 2005 – August 2009	28		25		17.8	1840*	7714	1701		Griessler et al. (2010)	Co-occurrence with DON, ZEA, aflatoxins, OTA	$FB_T = FB_1 + FB_2$
Lithuania		Breakfast cereals	2009	1	6	21		100.0					EFSA		

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Lithuania		Breakfast cereals	2010	2	6	21		50.0	156		156	156			
Lithuania		Barley	2009	1	6	21		0.0	931		931	931	EFSA		
Lithuania		Maize	2009	4	6	21		50.0	74		74	90			
Lithuania		Maize	2010	4	6	21		0.0	58.8		49	92			
Lithuania		Compound feedingstuffs	2009	2	6	21		0.0	5720		5720	6800			
Lithuania		Compound feedingstuffs	2010	1	6	21		100.0							
Luxembourg		Grains for human consumption	2009	4		214	0.0	75.0	89		89	89	EFSA		
Luxembourg		Grain milling products	2009	12	40	200	8.3	41.7	345.4		350.5	656.1			
Luxembourg		Pasta (raw)	2009	4		80	0.0	25.0	250.5		168	418.3			
Luxembourg		Breakfast cereals	2009	20	40	120	45.0	35.0	114.8		114.7	144.9			
Netherlands		Wheat middlings	2003	1	100		100.0						EFSA		
Netherlands		Wheat middlings	2004	1	100		100.0								

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Netherlands		Wheat middlings	2005	1	100		100.0								
Portugal		Yellow maize	2005	9					421	1061			Silva et al. (2007)		
Portugal		White maize	2005	2					638	1162					
Portugal		Maize flour	2005	3					995	2026					
Portugal		Maize semolina	2005	3					118	183					
Portugal		Maize starch	2005	3											
Portugal		Sweet maize	2005	11					64	523					
Portugal		Corn flakes	2005	16											
Portugal		Mixed cereals	2005	4											
Portugal		Maize snacks	2005	16					16	260					
Portugal	Coimbra	Maize bread (broa)	2005	30	35		16.7		274	550	266		Lino et al. (2007)		
Portugal		Feed	January 2005 – August 2009	10		25		30.0	631	3093	224		Griessler et al. (2010)	Co-occurrence with DON, ZEA, aflatoxins, OTA	$FB_T = FB_1 + FB_2$
Slovakia		Grains for human consumption	2008	2	80	250		100.0					EFSA		

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Slovakia		Grain milling products	2008	13	80	250		53.8	416.8		400	645			
Slovakia		Grain milling products	2009	2	20	50									
Slovakia		Bread and rolls	2008	3	30	100		33.3	59.1		59.1	86			
Slovakia		Fruiting vegetables	2008	10	80	250		30.0	384.9		204	1207.4			
Slovakia		Fruiting vegetables	2009												
Slovakia		Snack food	2008	4	30	100		75.0	140		140	140			
Slovakia		Snack food	2009												
Slovakia		Grains as crops	2008	5	80	250	0.0	60.0	202		202	270			
Slovakia		Maize	2008	2	80	250	0.0	100.0					EFSA		
Spain		Breakfast cereal	2009	4		200	0.0	100.0					EFSA		
Spain		Fine bakery wares	2009	4		200	0.0	100.0							
Spain		Whole corn	October 2002 – December 2004	92	25		0.0		2610	10 613			Castells et al. (2008)	Co-occurrence data available	

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Spain		Cornmeal derived from whole corn	October 2002 – December 2004	90	25		0.0		762	2003				Co-occurrence data available	
Spain		Corn flour derived from whole corn	October 2002 – December 2004	90	25		0.0		2640	6307				Co-occurrence data available	
Spain		Corn flaking grits derived from whole corn	October 2002 – December 2004	78	25		0.0		366	1053				Co-occurrence data available	
Spain		Cooked corn grits derived from whole corn	October 2002 – December 2004	47	25		87.0		140	258				Co-occurrence data available	
Spain		Corn flakes derived from whole corn	October 2002 – December 2004	47	25		79.0		42	67				Co-occurrence data available	
Europe and Mediterranean region		Feed grain (maize)	October 2003 – December 2005	16		100		43.8	836	2174	490		Binder et al. (2007)	Co-occurrence data for DON, ZEA, AFB_1, OTA	

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Europe and Mediterranean region		Feed grain (wheat)	October 2003 – December 2005	1		100		0.0	580	580	580			Co-occurrence data for DON, ZEA, AFB_1, OTA	
Europe and Mediterranean region		Feed grain (barley)	October 2003 – December 2005	1		100		100.0						Co-occurrence data for DON, ZEA, AFB_1, OTA	
Europe and Mediterranean region		Feed grain (oat)	October 2003 – December 2005	1		100		100.0						Co-occurrence data for DON, ZEA, AFB_1, OTA	
Europe and Mediterranean region		Finished feed	October 2003 – December 2005	10		100		70.0	638	1077	650			Co-occurrence data for DON, ZEA, AFB_1, OTA	
Europe and Mediterranean region		Soya bean meal	October 2003 – December 2005	2		100		50.0	3120	3120	3120			Co-occurrence data for DON, ZEA, AFB_1, OTA	

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Europe and Mediterranean region		Other feed ingredients	October 2003 – Decem-ber 2005	18		100		61.1	229	530	121			Co-occurrence data for DON, ZEA, AFB_1, OTA	
Europe		Other feed ingredients	2006	3	50			66.7	25	25			Submitted to JECFA		$FB_T = FB_1 + FB_2$
Europe		Other feed ingredients	2006	3	50			33.3	899.5	1752					$FB_T = FB_1 + FB_2$
Europe		Other feed ingredients	2006	3	50			100.0							
Europe		Maize (feed)	2006	4	50			0.0	308.5	770					$FB_T = FB_1 + FB_2$
Europe		Maize (feed)	2006	1	50		100.0								
Europe		Maize (feed)	2006	15		250		20.0	15 000	36 390					
Europe		Maize (feed)	2006	2		250		50.0	18 346	18 346					
Europe		Soya bean	2006	3		250		66.7	373	373					
Europe		Soya bean	2006	2	50		100.0								
Europe		Maize (feed)	2007	5	50		0.0		2303.2	7244					$FB_T = FB_1 + FB_2$

Table A1-4 (contd)

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence								References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Europe		Other feed ingredients	2007	5	50		0.0		999.8	3093					$FB_T = FB_1 + FB_2$
Europe		Other feed ingredients	2007	4	50		0.0		710.5	769					$FB_T = FB_1 + FB_2$
Europe		Other feed ingredients	2008	27	50		0.0		2144.8	11 246					$FB_T = FB_1 + FB_2$
Europe		Other feed ingredients	2008	4	50		50.0		456.5	502					$FB_T = FB_1 + FB_2$
Europe		Finished feed	2008	3	50		66.7		118	118					$FB_T = FB_1 + FB_2$
Europe		Finished feed	2008	6	50		33.3		1658.3	5213					$FB_T = FB_1 + FB_2$
Europe		Maize (feed)	2009	6	50		0.0		3432	7714					$FB_T = FB_1 + FB_2$
Europe		Maize (feed)	2009	6	25	80	0.0		2116.5	4013					$FB_T = FB_1 + FB_2$
Europe		Maize (feed)	2009	8	50		50.0		298.9*	939					$FB_T = FB_1 + FB_2$
Europe		Cereal	2009	13	50		69.2		385.6	2261					$FB_T = FB_1 + FB_2$

Country of sampling	Region	Food or feed commodity or their ingredients	Year	FB_T occurrence									References	Data on co-occurrence	Observations
				N	LOD (µg/kg)	LOQ (µg/kg)	% < LOD	% < LOQ	Mean (µg/kg)	Maximum (µg/kg)	Median (µg/kg)	90th percentile (µg/kg)			
Europe		Other feed ingredients	2009	6	50		100.0								$FB_T = FB_1 + FB_2$
Europe		Finished feed	2009	16	50		6.2		298.3	570					$FB_T = FB_1 + FB_2$
Europe		Finished feed	2009	3	50		33.3		971	1078					$FB_T = FB_1 + FB_2$
Europe		Finished feed	2009	4	50		100.0								
Europe		Finished feed	2009	2	50		100.0								
Europe		Finished feed	2009	2	50		0.0		1628	2282					$FB_T = FB_1 + FB_2$
Europe		Breeder layer mash	2009	2	50		0.0		573	930r					$FB_T = FB_1 + FB_2$

$FB_T = FB_1 + FB_2 + FB_3$ unless otherwise stated; AFB_1, aflatoxin B_1; DDGS, dried distillers' grains with solubles; DON, deoxynivalenol; EFSA, European Food Safety Authority; LOD, limit of detection; LOQ, limit of quantification; OTA, ochratoxin A; ZEA, zearalenone

* = Mean of all samples where non-detects are assigned a value of zero.

Table A1-5. Results of surveys of co-occurrence for fumonisins and aflatoxins showing concentrations and distribution of contamination in food and commodities

Country	Commodity or food	Year	*N*	Fumonisins (FB_T)				Aflatoxins (AF_T)				References	Observations
				A	B	M	Mx	A	B	M	Mx		
Brazil	"Canjica"	1999–2001	9	2.4/12	0/	190	530	1/	88.8/	1.7	16	Kawashima & Valente Soares (2006)	Only FB_1 analysed
Brazil	Corn flour	1999–2001	10	2.4/12	16.6/	61	150	1/	100/	0	0		$AF_T = AFB_1 + AFB_2$
Brazil	Corn flakes	1999–2001	31	2.4/12	0/	370	870	1/	96.5/	0.11	20		$AF_T = AFB_1 + AFB_2$
Brazil	Cornmeal	1999–2001	11	2.4/12	9/	2400	8600	1/	100/	0	0		$AF_T = AFB_1 + AFB_2$
Brazil	Popcorn	1999–2001	1	2.4/12	0/	21	21	1/	100/	0	0		$AF_T = AFB_1 + AFB_2$
Brazil	"Quierera"	1999–2001	6	2.4/12	0/	410	1400	1/	66.6/	5.9	20		$AF_T = AFB_1 + AFB_2$
Brazil	"Quierera fina"	1999–2001	6	2.4/12	0/	230	400	1/	83.3/	2	11		$AF_T = AFB_1 + AFB_2$
Brazil	Corn	2003	90	62.8/	0/	2540	15 320	4/	91.1/	24.1	54	Moreno et al. (2009)	$FB_T = FB_1 + FB_2$
Brazil	Corn	2003	60	62.8/	0/	3120	18 780	4/	83.3/	23.4	56		$FB_T = FB_1 + FB_2$
Brazil	Corn	2004	90	62.8/	1.1/	1310	18 160	4/	98.9/	0.4	40		$FB_T = FB_1 + FB_2$
Brazil	Corn	2004	60	62.8/	5/	1360	6280	4/	91.7/	35.2	52		$FB_T = FB_1 + FB_2$
Brazil	Corn	2005	50	/15	/8	920	8440	/2		28.8	976	Rocha et al. (2009)	
Brazil	Corn	2005	50	/15	/0	870	6270	/2		55.4	1393		
Brazil	Corn	2005	50	/15	/0	3770	9420	/2		2.1	48		
Brazil	Corn	2005	50	/15	/0	3760	9670	/2		0.7	34		
Guatemala	Incaparina	2000	3			800	1700			8	15	Trucksess et al. (2002)	$AF_T = AFB_1 + AFB_2$

Country	Commodity or food	Year	*N*	Fumonisins (FB_T)				Aflatoxins (AF_T)				References	Observations
				A	B	M	Mx	A	B	M	Mx		
Turkey	Maize	2002–2003	19		0/	88 240	356 800		0/	10.94	32.3	Oruc, Cengiz & Kalkanli (2006)	
Turkey	Maize	2002–2003	7		0/	74 200	263 000		0/	0.78	1.5		
Viet Nam	Maize	2008	5	/100	/60	350	1150	/2.5	/0	8.16	14.9	Trung et al. (2008)	Only FB_1 and AFB_1 analysed
Viet Nam	Maize	2008	3	/100	/100	0	0	/2.5	/0	14.4	11.3		Only FB_1 and AFB_1 analysed
Viet Nam	Maize	2008	5	/100	/80	100	500	/2.5	/60	8.7	12.3		Only FB_1 and AFB_1 analysed
Congo (Brazaville)	Cassava chips		6	1/	100/	0	0	1/			4.38	Manjula et al. (2009)	Only FB_1 and AFB_1 analysed
Congo (Brazaville)	Cassava flour		3	1/	100/	0	0	1/			1.64		Only FB_1 and AFB_1 analysed
Congo (Brazaville)	Maize		2	1/	100/	0	0	1/			71.67		Only FB_1 and AFB_1 analysed
Congo (Brazaville)	White maize		4	1/			1680	1/			10.81		Only FB_1 and AFB_1 analysed
Congo (Brazaville)	Mixed maize		4	1/			9620	1/			120.09		Only FB_1 and AFB_1 analysed
Ghana	Maize	1999	75	30/	9.3/	412.1	2534	0.2/	50.7/	231.9	1997.6	Kpodo (2001)	
Ghana	Processed maize (kenkey)	1999	75	30/	26.7/	228.1	1035	0.2/	22.7/	46.2	524.2		
Nigeria	Pre-harvest maize	2001	103	50/	21.4/		2010		81.6/	28	138	Bankole & Mabekoje (2004)	

Table A1-5 (contd)

Country	Commodity or food	Year	*N*	Fumonisins (FB_T)				Aflatoxins (AF_T)				References	Observations
				A	B	M	Mx	A	B	M	Mx		
Spain	Whole corn	2002–2004	92	25/	0/	2610	10 613	1.75/	65/	2.6	4.81	Castells et al. (2008)	
Spain	Cornmeal	2002–2004	90	25/	0/	762	2003	1.75/	97/	3.6	5.04		
Spain	Corn flour	2002–2004	90	25/	0/	2640	6307	1.75/	88/	4.9	7.5		
Spain	Corn flaking grits	2002–2004	78	25/	0/	366	1053	1.75/	83/	2.7	4.6		
Spain	Cooked corn grits	2002–2004	47	25/	87/	140	258	1.75/	89/	2.8	4.8		
Spain	Corn flakes	2002–2004	47	25/	79/	42	67	1.75/	81/	3.6	5.3		

$FB_T = FB_1 + FB_2 + FB_3$ unless otherwise stated; $AF_T = AFB_1 + AFB_2 + AFG_1 + AFG_2$ unless otherwise stated; *N* = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-6. Results of surveys of co-occurrence for fumonisin B_1, aflatoxin B_1 and ochratoxin A showing concentrations and distribution of contamination in food and commodities

Country	Commodity or food	Year	*N*	FB_1				AFB_1				OTA				Reference
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
Republic of Korea	Barley	1998–1999	30	5/	100/	0	0	1/	100/	0	0	1/	100/	0	0	Park et al. (2002)
Republic of Korea	Corn	1998–1999	18	5/	100/	0	0	1/	100/	0	0	1/	100/	0	0	
Republic of Korea	Barley foods	1998–1999	32	5/	94/	1	16	1/	88/	3.25	35	1/	88/	0.84	11	
Republic of Korea	Corn foods	1998–1999	47	5/	81/	14.2	122	1/	92/	1.7	25	1/	100/	0	0	

N = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-7. Results of surveys of co-occurrence for fumonisins, aflatoxins, deoxynivalenol and zearalenone showing concentrations and distribution of contamination in food and commodities

Country	Commodity	Year	*N*	Fumonisins (FB_T)				Aflatoxins (AF_T)				DON				ZEA				References
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
Argentina	Corn grain	2003	14	30/	16.7/	10 200	34 700	0.2/0.4	50/	3.2	11.3	9/12	66.7/	118.5	834	25/50	21.4/	230.8	2564	Broggi et al. (2007)
Argentina	Corn grain	2004	17	30/	29.4/	4700	16 100	0.2/0.4	41.2/	2.0	22.4	9/12	100/	0	0	25/50	100/	0	0	
Cameroon	Maize	2004–2005	12	10/	33.3/	940	3309	0.1/	41.7/	2.6	15	8/	50/	66	273	16/	25/	97	273	Njobeh et al. (2010)
Cameroon	Maize	2004–2005	7	10/	0/	7252	24 225	0.1/	42.8/	1.9	7	8/	28.6/	39	76	16/	57.1/	34	43	
Cameroon	Maize	2004–2005	12	10/	58.3/	4141	22 502	0.1/	41.7/	0.4	1.4	8/	8.3/	45	158	16/	8.3/	49	76	
Cameroon	Maize	2005	9	10/	33.3/	2396	5617	0.1/	55.6/	0.9	3.8	8/	22.2/	84	132	16/	11.1/	95	202	
Cameroon	Peanuts	2004–2005	3	10/	100/	0	0	0.1/	33.3/	5.4	10.6	8/	66.7/	270		16/	66.7/	51		
Cameroon	Peanuts	2004–2005	5	10/	80/	29		0.1/	0/	6.8	10.9	8/	0/	54	138	16/	20/	95	186	
Cameroon	Peanuts	2004–2005	4	10/	75/	25		0.1/	50/	5.5	10.7	8/	25/	59	167	16/	25/	87	160	
Cameroon	Peanuts	2005	4	10/	75/	1498		0.1/	25/	8.1	12.8	8/	25/	108	217	16/	50/	45	59	
Cameroon	Beans	2004–2005	3	10/	66.7/	764		0.1/	100/	0	0	8/	100/	0		16/	66.7/	57		

Table A1-7 (contd)

Country	Commodity	Year	*N*	Fumonisins (FB_T)				Aflatoxins (AF_T)				DON				ZEA				References
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
Cameroon	Beans	2004–2005	2	10/	100/	0		0.1/	100/	0	0	8/	50/	24		16/	100/			
Cameroon	Beans	2004–2005	10	10/	80/	690	1351	0.1/	50/	2.4	6.2	8/	40/	26	35	16/	60/	39	81	
Cameroon	Soya beans	2004–2005	1	10/	100/	0		0.1/	0/	3.9		8/	100/	0		16/	100/			
Cameroon	Soya beans	2004–2005	2	10/	50/	25		0.1/	100/	0		8/	100/	0		16/	100/			
Cameroon	Soya beans	2004–2005	2	10/	50/	365		0.1/	50/	0.2		8/	0/	110	207	16/	100/			

$FB_T = FB_1 + FB_2 + FB_3$; $AF_T = AFB_1 + AFB_2 + AFG_1 + AFG_2$; *N* = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-8. Results of surveys of co-occurrence for fumonisin B_1 plus fumonisin B_2 and deoxynivalenol showing concentrations and distribution of contamination in food

Country	Food	Year	*N*	$FB_1 + FB_2$				DON				Reference
				A	B	M[a]	Mx	A	B	M[a]	Mx	
USA	Popcorn	2005	5	10/	20/	244	500	/100	/60	0	60	Dowd & Johnson (2010)
USA	Popcorn	2006	8	10/	0/	785	1680	/100	/62.5	13.8	110	
USA	Popcorn	2007	7	10/	0/	218.4	710	/100	/57.1	75.7	320	
USA	Popcorn	2008	7	10/	0/	312.8	820	/100	/100	500	1900	

N = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

[a] Average calculated with values greater than LOQ and 0 for the others.

Table A1-9. Results of surveys of co-occurrence for fumonisin B_1 plus fumonisin B_2, deoxynivalenol, zearalenone, ochratoxin A, HT-2 toxin and nivalenol in Canadian samples (1999–2001) showing concentrations and distribution of contamination in food

Food	*N*	$FB_1 + FB_2$				DON				ZEA				OTA				HT-2 toxin				NIV				Reference
		A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
Corn-based breakfast cereal	34	4/10	11.7/	114	1980	10/20	64.7/	30	420	1/3	79.4/	1.3	21	0.05/0.2	82.3/	0.02	0.15	20/50	100/	0	0	20/50	100/	0	0	Roscoe et al. (2008)
Multigrain breakfast cereals	24	4/10	54.2/	12	88	10/20	41.66/	80	770	1/3	69.4/	4.6	100	0.05/0.2	55.5/	0.15	1	20/50	97.2/	1.7	60	20/50	97.3/	1.7	60	
Oat-based breakfast cereals	19	4/10	73.7/	6	57	10/20	37/	20	80	1/3	88.8/	0.5	6.9	0.05/0.2	37/	0.38	1.4	20/50	100/	0	0	20/50	100/	0	0	
Rice-based breakfast cereals	29	4/10	93.1/	0.3	5	10/20	96.5/	1.4	40	1/3	93.1/	0.2	3.6	0.05/0.2	89.6/	0.01	0.22	20/50	100/	0	0	20/50	100/	0	0	
Wheat-based breakfast cereals	29	4/10	82.7/	3	51	10/20	27.5/	110	940	1/3	62/	0.9	5.5	0.05/0.2	62.2/	0.11	0.64	20/50	100/	0	0	20/50	100/	0	0	
Buckwheat cereals	1	4/10	0/	5	5	10/20	100/	0	0	1/3	100/	0	0	0.05/0.2	100/	0	0	20/50	100/	0	0	20/50	100/	0	0	

N = number of samples; A = LOD /LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-10. Results of surveys of co-occurrence for fumonisins, deoxynivalenol, zearalenone, ochratoxin A, ergot alkaloids and T-2 toxin showing concentrations and distribution of contamination in food

Country	Food	Year	*N*	FB_T				DON				ZEA				OTA				Ergot alkaloids				T-2 toxin				Reference
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
Canada	Oat-based cereals	1997–1999	5	/20	/100	0	0	/20	/37.7	32	90	/3	/100	0	0	/0.2	/66.7	0.12	0.4	/4	/66.7	2	5	/4	/66.7	2	5	Lombaert et al. (2003)
Canada	Barley-based cereals	1997–1999	10	/20	/100	0	0	/20	/42	150	980	/3	/86.2	1.4	22	/0.2	/78.7	0.21	6.9	/4	/43.6	18	108	/4	/43.6	18	108	
Canada	Soya-based cereals	1997–1999	24	/20	/25	33	130	/20	/0	116	240	/3	/23.3	14.1	35	/0.2	/68.2	0.15	0.9	/4	/100	0	0	/4	/100			
Canada	Rice-based cereals	1997–1999	25	/20	/96	0	10	/20	/100	0		/3	/85.7	0.1	1	/0.2	/87.5	0.3	2.4	/4	/100	0	0	/4	/100			
Canada	Multigrain cereals	1997–1999	69	/20	/68.1	14	120	/20	/27.9	83	400	/3	/57.7	4.5	32	/0.2	/70.8	0.12	0.9	/4	/92	1	47	/4	/92	1	47	
Canada	Teething biscuits	1997–1999	2	/20	/50	10	20	/20	/25	45	120	/3	/85.7	1.2	8	/0.2	/80	0.06	0.3	/4	/77.8	1	4	/4	/77.8	1	4	
Canada	Soya formulas	1997–1999	1	/20	/100	0	0	/20	/100	0		/3	/100	0	0	/0.2	/100	0	0	/4	/100	0	0	/4	/100			
Canada	Creamed corn	1997–1999	6	/20	/100	0	0	/20	/100	0		/3	/100	0	0													

$FB_T = FB_1 + FB_2 + FB_3$; *N* = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-11. Results of surveys of co-occurrence for fumonisins, aflatoxins, deoxynivalenol, zearalenone, ochratoxin A and T-2 toxin showing concentrations and distribution of contamination in food commodities

Country	Commodity	Year	*N*	FB_T				AF_T				DON				ZEA				OTA				T-2 toxin				Reference
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
China, Tibet Autonomous Region	Barley	1997	25	200/	44/	450	1100	2/	96/	40		100/	88/	161	200	5/	4/	161	270	1/	48/	14	46	25/	24/	39	163	Haubruge et al. (2003)

$FB_T = FB_1 + FB_2 + FB_3$; $AF_T = AFB_1 + AFB_2 + AFG_1 + AFG_2$; *N* = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-12. Results of surveys of co-occurrence for fumonisin B_1, aflatoxin B_1, deoxynivalenol, zearalenone, ochratoxin A and nivalenol showing concentrations and distribution of contamination in food commodities

Country	Commodity	Year	*N*	FB_1				AFB_1				DON				ZEA				OTA				NIV				Reference
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
Republic of Korea	Rice	2002	88	35/	97.7/	1.2	60.6	1/	94.3/	0.24	7.3	100/	96.6/	4.7	159	4/	96.6/	1.3	47	1/	90.9/	0.35	6	100/	94.3/	20	462	Park et al. (2005)

N = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-13. Results of surveys of co-occurrence for fumonisin B_1 and ochratoxin A showing concentrations and distribution of contamination in food

Country	Food	Year	*N*	FB_1				OTA				Reference
				A	B	M	Mx	A	B	M	Mx	
France	Breakfast cereals		32	1/2	6.2/12.5	79.2	1113	0.05/0.2	31.1/53.3	1.27	8.8	Molinié et al. (2005)

N = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-14. Results of surveys of co-occurrence for fumonisin B_1 plus fumonisin B_2, deoxynivalenol and zearalenone showing concentrations and distribution of contamination in food commodities

Country	Commodity	Year	*N*	$FB_1 + FB_2$				DON				ZEA				Reference
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
Germany	Maize	2006	44	/150	/22.7	1910	20 690	/40	/25	1780	19 570	/3	/73	70	860	Goertz et al. (2010)
Germany	Maize	2007	40	/150	/100	0	0	/40	/10	2240	16 250	/3	/7	480	14 580	

N = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-15. Results of surveys of co-occurrence for fumonisin B_1, zearalenone and ochratoxin A showing concentrations and distribution of contamination in food commodities

Country	Commodity	Year	*N*	FB_1				ZEA				OTA				Reference
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
Morocco	Corn		10	30/60	0/	1930	5960	5/10	70/	14.3	17	0.01/0.02	20/	1.08	7.22	Zinedine et al. (2006)

N = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-16. Results of surveys of co-occurrence for fumonisins, aflatoxins, deoxynivalenol, zearalenone and T-2 toxin showing concentrations and distribution of contamination in feeds

Country	Feed	Year	*N*	FB_T				AF_T				DON				ZEA				T-2 toxin				References
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
USA	Feed (DDGS)	2007–2008	20	100/	5/	1880	8600	0.5/	70/	0.66	3.7	100/	25/	460	1100	100/	55/	38.2	143	300/	100/	0	0	Zhang et al. (2009)
USA	Feed (DDGS)	2006–2007	69	100/	0/	2327	5880	0.5/	95.6/	0.08	2.56	100/	4.3/	665.3	1420	50/	68.1/	24.9	123	100/	100/	0	0	Zhang et al. (2009)
Americas	Corn, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2006–2007	48	100/	37/	1106	7910	4/	94/	244	709	50/	50/	906	2708	32/	67/	114	291	125/	98/	133	133	Rodrigues (2008)
Asia	Corn, soya bean meal, wheat/bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/silage	2006–2007	269	100/	46/	1002	10 766	4/	54/	61	1023	50/	85/	279	1130	32/	67/	209	4011	125/	100/	0	0	Rodrigues (2008)

DDGS, dried distillers' grains with solubles

$FB_T = FB_1 + FB_2 + FB_3$; $AF_T = AFB_1 + AFB_2 + AFG_1 + AFG_2$; *N* = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-17. Results of surveys of co-occurrence for fumonsins and aflatoxins showing concentrations and distribution of contamination in feeds

Country	Feed	Year	*N*	FB_T				AF_T				Reference
				A	B	M	Mx	A	B	M	Mx	
Viet Nam	Maize (feed)	2008	5	/100	/80	660	3300	/2.5	/40	48	126.5	Trung et al. (2008)
Viet Nam	Maize (feed)	2008	3	/100	/100	0	0	/2.5	/66.7	7	21.1	
Viet Nam	Maize (feed)	2008	4	/100	/0	855	1120	/2.5	/50	24.3	47.2	

$FB_T = FB_1 + FB_2 + FB_3$; $AF_T = AFB_1 + AFB_2 + AFG_1 + AFG_2$; *N* = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-18. Results of surveys of co-occurrence for fumonisins, aflatoxins, zearalenone and ochratoxin A showing concentrations and distribution of contamination in feeds

Country	Feed	Year	*N*	FB_T				AF_T				ZEA				OTA				References
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
Argentina	Pig feed (sow)	2008	50	1/		334.2		1/	20/	228.2		50/	100/	0		0.1/	100/	0		Pereyra et al. (2010)
Argentina	Pig feed (non-pregnant gilt)	2008	50	1/		353.1		1/	100/	0		50/	100/	0		0.1/	0/	0.259		
Argentina	Pig feed (pregnant gilt)	2008	50	1/		341.6		1/	100/	0		50/	100/	0		0.1/	100/	0		
South Africa	Animal feed	—	23	5/	73.9/	1188.8	5900	4/	26.1/	39.7	156	4/	87/	55 867	165 000	6/	100/			Mngadi, Govinden & Odhav (2008)
Croatia	Feed	2007	13	200/	46.2/	2300	5000	2/	69.2/	6.9	10.3	10/	0/	626.6	1168	1/	84.6/	9.2	12.9	Klarić et al. (2009)

$FB_T = FB_1 + FB_2 + FB_3$; $AF_T = AFB_1 + AFB_2 + AFG_1 + AFG_2$; *N* = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-19. Results of surveys of co-occurrence for fumonisin B_1, deoxynivalenol, zearalenone, ochratoxin A and ergot alkaloids showing concentrations and distribution of contamination in feeds

Country	Feed	Year	*N*	FB_1				DON				ZEA				OTA				Ergot				References
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
Germany	Commercial horsefeed preparations	2007–2008	62	2/	6/	100	2200	10/	0/	410	4900	5/	2/	78	310	0.2/	58/	0.66	4	30/	39/	140	1200	Liesener et al. (2010)

N = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-20. Results of surveys of co-occurrence for fumonisins, deoxynivalenol and zearalenone showing concentrations and distribution of contamination in feeds

Country	Feed	Year	*N*	FB_T				DON				ZEA				References
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
Portugal	Mixed feed for laying hens		52	150/	80.8/	73.6	110	100/	86.5/	118.1	253	5/	69.3/	27	61.4	Martins et al. (2006)

$FB_T = FB_1 + FB_2 + FB_3$; *N* = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-21. Results of surveys of co-occurrence for fumonisins, aflatoxins, deoxynivalenol, zearalenone and ochratoxin A showing concentrations and distribution of contamination in feeds

Country	Feed	Year	*N*	FB_T				AF_T				DON				ZEA				OTA				Reference
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
Asia	Corn, soya bean meal, wheat/ bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/ silage	2010	369	100/	47/	493	6196	4/	35/	22	726	50/	59/	299	19 096	32/	51/	55	2601	2/	68/	1	53	Rodrigues & Naehrer (2011a)
Asia	Corn, soya bean meal, wheat/ bran, corn gluten meal, rice/bran, DDGS, feed ingredients, finished feed, straw/ silage	2010	89	100/	46/	323	1852	4/	12/	78	593	50/	70/	47	556	32/	70/	14	297	2/	29/	9	174	

DDGS, dried distillers' grains with solubles

$FB_T = FB_1 + FB_2 + FB_3$; $AF_T = AFB_1 + AFB_2 + AFG_1 + AFG_2$; *N* = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-22. Results of surveys of co-occurrence for fumonisins, aflatoxins, deoxynivalenol, zearalenone and T-2 toxin showing concentrations and distribution of contamination in feeds

Country	Feed	Year	*N*	FB_T				AF_T				ZEA				T-2 Toxin				Reference
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
USA	Feed (DDGS from China, Province of Taiwan)	2006	7	100/	0/	2300	2900	5/	100/	0	0	500/	100/	0	0	100/	100/	0	0	Zhang et al. (2009)
USA	Feed (DDGS from China, Province of Taiwan)	2006	11	100/	0/	1550	2400	5/	100/	0	0	500/	100/	0	0	100/	100/	0	0	
USA	Feed (DDGS from China, Province of Taiwan)	2007	24	100/	0/	1200	2400	5/	100/	0	0	500/	100/	0	0	100/	100/	0	0	
USA	Feed (DDGS)	2006–2007	16	100/	0/	1471	2770	0.5/	87.5/	0.15	1.21	100/	43.75/	42.38	113	100/	100/	0	0	

DDGS, dried distillers' grains with solubles

$FB_T = FB_1 + FB_2 + FB_3$; $AF_T = AFB_1 + AFB_2 + AFG_1 + AFG_2$; *N* = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-23. Results of surveys of co-occurrence for fumonisin B_1, aflatoxins and zearalenone showing concentrations and distribution of contamination in feeds

Country	Feed	Year	*N*	FB_1				AF_T				ZEA				Reference
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
Brazil	Poultry feeds	2003–2004	480	2/	2.2/		5500	1/	33.3/		17.5		22.9/		7000	Oliveira et al. (2006)

AF_T = AFB_1 + AFB_2 + AFG_1 + AFG_2; *N* = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-24. Results of surveys of co-occurrence for fumonisins, aflatoxins, deoxynivalenol and zearalenone showing concentrations and distribution of contamination in feeds

Country	Feed	Year	N	FB_T				AF_T				Don				ZEA				Reference
				A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	A	B	M	Mx	
USA	Feed (DDGS)	2008	77	100/200	/2.6	2687	7200	3/	100/	0	0	100/	0/	468.4	1900	200/	100/	0	0	Zhang et al. (2009)

DDGS, dried distillers' grains with solubles

FB_T = FB_1 + FB_2 + FB_3; AF_T = AFB_1 + AFB_2 + AFG_1 + AFG_2; *N* = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average (µg/kg); Mx = Maximum (µg/kg)

Table A1-25. Occurrence of hidden and bound fumonisins in food and commodities

Country of origin and sampling	Commodity or food	Year	*N*	A	B	Hidden and bound fumonisins occurrence (µg/kg)				References	Observations
						M	Mx	Median	90th percentile		
Canada	Corn flakes	2003	26	8/	11.5/	76.7	201	65	163.5	Kim, Scott & Lau (2003)	FB_1
Canada	Corn flakes	2003	26	8/	50/	9.2	31	4	26		FB_2
Canada	Corn flakes	2003	26	8/	0/	95.7	288	66	198.5		Bound fumonisin calculated as HFB_1 after hydrolysis
Canada	Corn flakes	2004	11	8/	18.1/	39.6	113	34	79	Park et al. (2004)	Protein-bound FB_1 extracted with 1% SDS after hydrolysis and measured as HFB_1
Canada	Corn flakes	2004	11	8/	9/	70.7	188	60	112		Total bound FB_1 extracted after hydrolysis and measured as HFB_1
Canada	Corn-based products	2004	4	8/	25/	65	176	42	141.8		Protein-bound FB_1 extracted with 1% SDS after hydrolysis and measured as HFB_1
Canada	Corn-based products	2004	4	8/	25/	151.2	418	93.5	332.2		Total bound FB_1 extracted after hydrolysis and measured as HFB_1
Canada	Tortilla chips	2004	13	8/	84.6/	6.5	46	0	31.2		Protein-bound FB_1 extracted with 1% SDS after hydrolysis and measured as HFB_1
Canada	Tortilla chips	2004	13	8/	61.5/	38.3	209	0	84.2		Total bound FB_1 extracted after hydrolysis and measured as HFB_1
Italy	Gluten-free snack	2008	9	8/	22.2/		554	354		Dall'Asta et al. (2009a)	$FB_1 + FB_2 + FB_3$ (LOD $FB_1 = 4$, $FB_2 + FB_3 = 8$)
Italy	Gluten	2008	9	20/	22.2/		245	89			HFBs ($HFB_1 + HFB_2 + HFB_3$); LOD $HFB_x = 20$

Country of origin and sampling	Commodity or food	Year	*N*	A	B	Hidden and bound fumonisins occurrence (µg/kg)				References	Observations
						M	Mx	Median	90th percentile		
Italy	Gluten	2008	4				4740	1430			Bound fumonisin calculated by subtracting free FB_1 and HFB_1 found before hydrolysis from total fumonisin content
Italy	Gluten-free pasta, bread	2008	17	8/	11.8/		513	66			$FB_1 + FB_2 + FB_3$ (LOD FB_1 = 4, $FB_2 + FB_3$ = 8)
Italy	Gluten-free pasta, bread	2008	17	20/70	11.8/		127	22			HFBs ($HFB_1 + HFB_2 + HFB_3$); LOD HFB_x = 20
Italy	Gluten-free flours	2008	7	8/70	0/		3310	1020			$FB_1 + FB_2 + FB_3$ (LOQ FB_1 = 5 , FB_2 =12, FB_3 = 70)
Italy	Gluten-free flours	2008	7	20/70	0/		621	259			HFBs ($HFB_1 + HFB_2 + HFB_3$); LOQ HFB_1 = 70, HFB_2 = 70, HFB_3 = 70
Italy	Gluten-free pasta, bread, flour	2008	11				1530	148			Bound fumonisin calculated by subtracting free FB_1 and HFB_1 found before hydrolysis from total fumonisin content
Italy	Gluten-free extruded products	2008	7	8/	42.9/		2250	39			$FB_1 + FB_2 + FB_3$ (LOD FB_1 = 4, FB_2 and FB_3 = 8)
Italy	Gluten-free extruded products	2008	7	20/	42.9/		458	31			HFBs ($HFB_1 + HFB_2 + HFB_3$); LOD HFB_x = 20

Table A1-25 (contd)

Country of origin and sampling	Commodity or food	Year	*N*	A	B	Hidden and bound fumonisins occurrence (µg/kg)				References	Observations
						M	Mx	Median	90th percentile		
Italy	Corn flakes	2008	6				245	89			Bound fumonisin calculated by subtracting free FB_1 and HFB_1 found before hydrolysis from total fumonisin content
Italy	Raw maize	2008	31	/10	/0	4808.5	17 014	3523	9689	Dall'Asta et al. (2010)	Extractable fumonisins
Italy	Raw maize	2008	31	/10	/0	8960.1	40 821	6074	21 112		Total fumonisins
Italy	Raw maize	2008	31	/10	/0	4163	23 807	1468	11 436		Hidden fumonisin calculated as difference among "total fumonisin" obtained after digestion analysis and "extractable fumonisins" obtained after routine analysis
Mexico	Nixtamalized cornmeal, grocery stores	2002	20	500/	0/	2570	3670			Cortez-Rocha et al. (2005)	HFB_1 calculated with immunoaffinity column method
Mexico	Nixtamalized cornmeal, supermarkets	2002	32	500/	37.5/	3610	13 710				HFB_1 calculated with immunoaffinity column method
Mexico	Nixtamalized cornmeal, retail outlets	2002	18	500/	5.6/	3610	4330				HFB_1 calculated with immunoaffinity column method

SDS, sodium dodecyl sulfate
N = number of samples; A = LOD/LOQ (µg/kg); B = % < LOD / % < LOQ; M = Average; Mx = Maximum

ANNEXES

ANNEX 1

REPORTS AND OTHER DOCUMENTS RESULTING FROM PREVIOUS MEETINGS OF THE JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

1. *General principles governing the use of food additives* (First report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 15, 1957; WHO Technical Report Series, No. 129, 1957 (out of print).
2. *Procedures for the testing of intentional food additives to establish their safety for use* (Second report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 17, 1958; WHO Technical Report Series, No. 144, 1958 (out of print).
3. *Specifications for identity and purity of food additives (antimicrobial preservatives and antioxidants)* (Third report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as *Specifications for identity and purity of food additives, Vol. I. Antimicrobial preservatives and antioxidants,* Rome, Food and Agriculture Organization of the United Nations, 1962 (out of print).
4. *Specifications for identity and purity of food additives (food colours)* (Fourth report of the Joint FAO/WHO Expert Committee on Food Additives). These specifications were subsequently revised and published as Specifications for identity and purity of food additives, Vol. II. Food colours, Rome, Food and Agriculture Organization of the United Nations, 1963 (out of print).
5. *Evaluation of the carcinogenic hazards of food additives* (Fifth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 29, 1961; WHO Technical Report Series, No. 220, 1961 (out of print).
6. *Evaluation of the toxicity of a number of antimicrobials and antioxidants* (Sixth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Report Series, No. 31, 1962; WHO Technical Report Series, No. 228, 1962 (out of print).
7. *Specifications for the identity and purity of food additives and their toxicological evaluation: emulsifiers, stabilizers, bleaching and maturing agents* (Seventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 35, 1964; WHO Technical Report Series, No. 281, 1964 (out of print).
8. *Specifications for the identity and purity of food additives and their toxicological evaluation: food colours and some antimicrobials and antioxidants* (Eighth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 38, 1965; WHO Technical Report Series, No. 309, 1965 (out of print).
9. *Specifications for identity and purity and toxicological evaluation of some antimicrobials and antioxidants*. FAO Nutrition Meetings Report Series, No. 38A, 1965; WHO/Food Add/24.65 (out of print).
10. *Specifications for identity and purity and toxicological evaluation of food colours.* FAO Nutrition Meetings Report Series, No. 38B, 1966; WHO/Food Add/66.25.

11. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases* (Ninth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 40, 1966; WHO Technical Report Series, No. 339, 1966 (out of print).
12. *Toxicological evaluation of some antimicrobials, antioxidants, emulsifiers, stabilizers, flour treatment agents, acids, and bases.* FAO Nutrition Meetings Report Series, No. 40A, B, C; WHO/Food Add/67.29.
13. *Specifications for the identity and purity of food additives and their toxicological evaluation: some emulsifiers and stabilizers and certain other substances* (Tenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 43, 1967; WHO Technical Report Series, No. 373, 1967.
14. *Specifications for the identity and purity of food additives and their toxicological evaluation: some flavouring substances and non nutritive sweetening agents* (Eleventh report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 44, 1968; WHO Technical Report Series, No. 383, 1968.
15. *Toxicological evaluation of some flavouring substances and non nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44A, 1968; WHO/Food Add/68.33.
16. *Specifications and criteria for identity and purity of some flavouring substances and non-nutritive sweetening agents.* FAO Nutrition Meetings Report Series, No. 44B, 1969; WHO/Food Add/69.31.
17. *Specifications for the identity and purity of food additives and their toxicological evaluation: some antibiotics* (Twelfth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 45, 1969; WHO Technical Report Series, No. 430, 1969.
18. *Specifications for the identity and purity of some antibiotics.* FAO Nutrition Meetings Series, No. 45A, 1969; WHO/Food Add/69.34.
19. *Specifications for the identity and purity of food additives and their toxicological evaluation: some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances* (Thirteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 46, 1970; WHO Technical Report Series, No. 445, 1970.
20. *Toxicological evaluation of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other substances.* FAO Nutrition Meetings Report Series, No. 46A, 1970; WHO/Food Add/70.36.
21. *Specifications for the identity and purity of some food colours, emulsifiers, stabilizers, anticaking agents, and certain other food additives.* FAO Nutrition Meetings Report Series, No. 46B, 1970; WHO/Food Add/70.37.
22. *Evaluation of food additives: specifications for the identity and purity of food additives and their toxicological evaluation: some extraction solvents and certain other substances; and a review of the technological efficacy of some antimicrobial agents.* (Fourteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 48, 1971; WHO Technical Report Series, No. 462, 1971.
23. *Toxicological evaluation of some extraction solvents and certain other substances.* FAO Nutrition Meetings Report Series, No. 48A, 1971; WHO/Food Add/70.39.

24. *Specifications for the identity and purity of some extraction solvents and certain other substances*. FAO Nutrition Meetings Report Series, No. 48B, 1971; WHO/Food Add/70.40.
25. *A review of the technological efficacy of some antimicrobial agents.* FAO Nutrition Meetings Report Series, No. 48C, 1971; WHO/Food Add/70.41.
26. *Evaluation of food additives: some enzymes, modified starches, and certain other substances: Toxicological evaluations and specifications and a review of the technological efficacy of some antioxidants* (Fifteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 50, 1972; WHO Technical Report Series, No. 488, 1972.
27. *Toxicological evaluation of some enzymes, modified starches, and certain other substances*. FAO Nutrition Meetings Report Series, No. 50A, 1972; WHO Food Additives Series, No. 1, 1972.
28. *Specifications for the identity and purity of some enzymes and certain other substances.* FAO Nutrition Meetings Report Series, No. 50B, 1972; WHO Food Additives Series, No. 2, 1972.
29. *A review of the technological efficacy of some antioxidants and synergists.* FAO Nutrition Meetings Report Series, No. 50C, 1972; WHO Food Additives Series, No. 3, 1972.
30. *Evaluation of certain food additives and the contaminants mercury, lead, and cadmium* (Sixteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 51, 1972; WHO Technical Report Series, No. 505, 1972, and corrigendum.
31. *Evaluation of mercury, lead, cadmium and the food additives amaranth, diethylpyrocarbamate, and octyl gallate*. FAO Nutrition Meetings Report Series, No. 51A, 1972; WHO Food Additives Series, No. 4, 1972.
32. *Toxicological evaluation of certain food additives with a review of general principles and of specifications* (Seventeenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 53, 1974; WHO Technical Report Series, No. 539, 1974, and corrigendum (out of print).
33. *Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidants, emulsifiers, and thickening agents.* FAO Nutrition Meetings Report Series, No. 53A, 1974; WHO Food Additives Series, No. 5, 1974.
34. *Specifications for identity and purity of thickening agents, anticaking agents, antimicrobials, antioxidants and emulsifiers*. FAO Food and Nutrition Paper, No. 4, 1978.
35. *Evaluation of certain food additives* (Eighteenth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 54, 1974; WHO Technical Report Series, No. 557, 1974, and corrigendum.
36. *Toxicological evaluation of some food colours, enzymes, flavour enhancers, thickening agents, and certain other food additives*. FAO Nutrition Meetings Report Series, No. 54A, 1975; WHO Food Additives Series, No. 6, 1975.
37. *Specifications for the identity and purity of some food colours, enhancers, thickening agents, and certain food additives*. FAO Nutrition Meetings Report Series, No. 54B, 1975; WHO Food Additives Series, No. 7, 1975.
38. *Evaluation of certain food additives: some food colours, thickening agents, smoke condensates, and certain other substances*. (Nineteenth report of the

Joint FAO/WHO Expert Committee on Food Additives). FAO Nutrition Meetings Series, No. 55, 1975; WHO Technical Report Series, No. 576, 1975.
39. *Toxicological evaluation of some food colours, thickening agents, and certain other substances*. FAO Nutrition Meetings Report Series, No. 55A, 1975; WHO Food Additives Series, No. 8, 1975.
40. *Specifications for the identity and purity of certain food additives*. FAO Nutrition Meetings Report Series, No. 55B, 1976; WHO Food Additives Series, No. 9, 1976.
41. *Evaluation of certain food additives* (Twentieth report of the Joint FAO/WHO Expert Committee on Food Additives). FAO Food and Nutrition Meetings Series, No. 1, 1976; WHO Technical Report Series, No. 599, 1976.
42. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 10, 1976.
43. *Specifications for the identity and purity of some food additives.* FAO Food and Nutrition Series, No. 1B, 1977; WHO Food Additives Series, No. 11, 1977.
44. *Evaluation of certain food additives* (Twenty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 617, 1978.
45. *Summary of toxicological data of certain food additives*. WHO Food Additives Series, No. 12, 1977.
46. Specifications for identity and purity of some food additives, including antioxidant, food colours, thickeners, and others. FAO Nutrition Meetings Report Series, No. 57, 1977.
47. *Evaluation of certain food additives and contaminants* (Twenty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 631, 1978.
48. *Summary of toxicological data of certain food additives and contaminants*. WHO Food Additives Series, No. 13, 1978.
49. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 7, 1978.
50. *Evaluation of certain food additives* (Twenty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 648, 1980, and corrigenda.
51. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 14, 1980.
52. *Specifications for identity and purity of food colours, flavouring agents, and other food additives*. FAO Food and Nutrition Paper, No. 12, 1979.
53. *Evaluation of certain food additives* (Twenty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 653, 1980.
54. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 15, 1980.
55. *Specifications for identity and purity of food additives (sweetening agents, emulsifying agents, and other food additives)*. FAO Food and Nutrition Paper, No. 17, 1980.
56. *Evaluation of certain food additives* (Twenty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 669, 1981.

57. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 16, 1981.
58. *Specifications for identity and purity of food additives (carrier solvents, emulsifiers and stabilizers, enzyme preparations, flavouring agents, food colours, sweetening agents, and other food additives)*. FAO Food and Nutrition Paper, No. 19, 1981.
59. *Evaluation of certain food additives and contaminants* (Twenty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 683, 1982.
60. *Toxicological evaluation of certain food additives.* WHO Food Additives Series, No. 17, 1982.
61. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 25, 1982.
62. *Evaluation of certain food additives and contaminants* (Twenty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 696, 1983, and corrigenda.
63. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 18, 1983.
64. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 28, 1983.
65. *Guide to specifications—General notices, general methods, identification tests, test solutions, and other reference materials*. FAO Food and Nutrition Paper, No. 5, Rev. 1, 1983.
66. *Evaluation of certain food additives and contaminants* (Twenty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 710, 1984, and corrigendum.
67. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 19, 1984.
68. *Specifications for the identity and purity of food colours*. FAO Food and Nutrition Paper, No. 31/1, 1984.
69. *Specifications for the identity and purity of food additives*. FAO Food and Nutrition Paper, No. 31/2, 1984.
70. *Evaluation of certain food additives and contaminants* (Twenty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 733, 1986, and corrigendum.
71. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 34, 1986.
72. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 20. Cambridge University Press, 1987.
73. *Evaluation of certain food additives and contaminants* (Thirtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 751, 1987.
74. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 21. Cambridge University Press, 1987.
75. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 37, 1986.
76. *Principles for the safety assessment of food additives and contaminants in food*. WHO Environmental Health Criteria, No. 70. Geneva, World Health

Organization, 1987 (out of print). The full text is available electronically at www.who.int/pcs.

77. *Evaluation of certain food additives and contaminants* (Thirty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 759, 1987 and corrigendum.
78. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 22. Cambridge University Press, 1988.
79. *Specifications for the identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 38, 1988.
80. *Evaluation of certain veterinary drug residues in food* (Thirty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 763, 1988.
81. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 23. Cambridge University Press, 1988.
82. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41, 1988.
83. *Evaluation of certain food additives and contaminants* (Thirty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 776, 1989.
84. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 24. Cambridge University Press, 1989.
85. *Evaluation of certain veterinary drug residues in food* (Thirty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 788, 1989.
86. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 25, 1990.
87. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/2, 1990.
88. *Evaluation of certain food additives and contaminants* (Thirty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 789, 1990, and corrigenda.
89. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 26, 1990.
90. *Specifications for identity and purity of certain food additives*. FAO Food and Nutrition Paper, No. 49, 1990.
91. *Evaluation of certain veterinary drug residues in food* (Thirty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 799, 1990.
92. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 27, 1991.
93. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/3, 1991.
94. *Evaluation of certain food additives and contaminants* (Thirty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 806, 1991, and corrigenda.
95. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 28, 1991.
96. *Compendium of food additive specifications (Joint FAO/WHO Expert Committee on Food Additives (JECFA)). Combined specifications from 1st through the*

37th meetings, 1956–1990. Rome, Food and Agricultural Organization of the United Nations, 1992 (2 volumes).

97. *Evaluation of certain veterinary drug residues in food* (Thirty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 815, 1991.
98. *Toxicological evaluation of certain veterinary residues in food*. WHO Food Additives Series, No. 29, 1991.
99. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/4, 1991.
100. *Guide to specifications—General notices, general analytical techniques, identification tests, test solutions, and other reference materials*. FAO Food and Nutrition Paper, No. 5, Rev. 2, 1991.
101. *Evaluation of certain food additives and naturally occurring toxicants* (Thirty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 828, 1992.
102. *Toxicological evaluation of certain food additives and naturally occurring toxicants*. WHO Food Additive Series, No. 30, 1993.
103. *Compendium of food additive specifications: addendum 1*. FAO Food and Nutrition Paper, No. 52, 1992.
104. *Evaluation of certain veterinary drug residues in food* (Fortieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 832, 1993.
105. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 31, 1993.
106. *Residues of some veterinary drugs in animals and food*. FAO Food and Nutrition Paper, No. 41/5, 1993.
107. *Evaluation of certain food additives and contaminants* (Forty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 837, 1993.
108. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 32, 1993.
109. *Compendium of food additive specifications: addendum 2*. FAO Food and Nutrition Paper, No. 52, Add. 2, 1993.
110. *Evaluation of certain veterinary drug residues in food* (Forty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 851, 1995.
111. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 33, 1994.
112. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/6, 1994.
113. *Evaluation of certain veterinary drug residues in food* (Forty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 855, 1995, and corrigendum.
114. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 34, 1995.
115. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/7, 1995.
116. *Evaluation of certain food additives and contaminants* (Forty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 859, 1995.

117. *Toxicological evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 35, 1996.
118. *Compendium of food additive specifications: addendum 3*. FAO Food and Nutrition Paper, No. 52, Add. 3, 1995.
119. *Evaluation of certain veterinary drug residues in food* (Forty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 864, 1996.
120. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 36, 1996.
121. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/8, 1996.
122. *Evaluation of certain food additives and contaminants* (Forty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 868, 1997.
123. *Toxicological evaluation of certain food additives*. WHO Food Additives Series, No. 37, 1996.
124. *Compendium of food additive specifications, addendum 4*. FAO Food and Nutrition Paper, No. 52, Add. 4, 1996.
125. *Evaluation of certain veterinary drug residues in food* (Forty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 876, 1998.
126. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 38, 1996.
127. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/9, 1997.
128. *Evaluation of certain veterinary drug residues in food* (Forty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 879, 1998.
129. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 39, 1997.
130. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/10, 1998.
131. *Evaluation of certain food additives and contaminants* (Forty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 884, 1999.
132. *Safety evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 40, 1998.
133. *Compendium of food additive specifications: addendum 5*. FAO Food and Nutrition Paper, No. 52, Add. 5, 1997.
134. *Evaluation of certain veterinary drug residues in food* (Fiftieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 888, 1999.
135. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 41, 1998.
136. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/11, 1999.
137. *Evaluation of certain food additives* (Fifty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 891, 2000.

138. *Safety evaluation of certain food additives*. WHO Food Additives Series, No. 42, 1999.
139. *Compendium of food additive specifications, addendum 6*. FAO Food and Nutrition Paper, No. 52, Add. 6, 1998.
140. *Evaluation of certain veterinary drug residues in food* (Fifty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 893, 2000.
141. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 43, 2000.
142. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/12, 2000.
143. *Evaluation of certain food additives and contaminants* (Fifty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 896, 2000.
144. *Safety evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 44, 2000.
145. *Compendium of food additive specifications, addendum 7*. FAO Food and Nutrition Paper, No. 52, Add. 7, 1999.
146. *Evaluation of certain veterinary drug residues in food* (Fifty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 900, 2001.
147. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 45, 2000.
148. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/13, 2000.
149. *Evaluation of certain food additives and contaminants* (Fifty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 901, 2001.
150. Safety evaluation of certain food additives and contaminants. WHO Food Additives Series, No. 46, 2001.
151. *Compendium of food additive specifications: addendum 8*. FAO Food and Nutrition Paper, No. 52, Add. 8, 2000.
152. *Evaluation of certain mycotoxins in food* (Fifty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series No. 906, 2002.
153. *Safety evaluation of certain mycotoxins in food*. WHO Food Additives Series, No. 47/FAO Food and Nutrition Paper 74, 2001.
154. *Evaluation of certain food additives and contaminants* (Fifty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 909, 2002.
155. *Safety evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 48, 2002.
156. *Compendium of food additive specifications: addendum 9*. FAO Food and Nutrition Paper, No. 52, Add. 9, 2001.
157. *Evaluation of certain veterinary drug residues in food* (Fifty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 911, 2002.
158. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 49, 2002.

159. *Residues of some veterinary drugs in animals and foods*. FAO Food and Nutrition Paper, No. 41/14, 2002.
160. *Evaluation of certain food additives and contaminants* (Fifty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 913, 2002.
161. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 50, 2003.
162. *Compendium of food additive specifications: addendum 10*. FAO Food and Nutrition Paper No. 52, Add. 10, 2002.
163. *Evaluation of certain veterinary drug residues in food* (Sixtieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 918, 2003.
164. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 51, 2003.
165. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/15, 2003.
166. *Evaluation of certain food additives and contaminants* (Sixty-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 922, 2004.
167. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 52, 2004.
168. *Compendium of food additive specifications: addendum 11.* FAO Food and Nutrition Paper, No. 52, Add. 11, 2003.
169. *Evaluation of certain veterinary drug residues in food* (Sixty-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 925, 2004.
170. *Residues of some veterinary drugs in animals and foods.* FAO Food and Nutrition Paper, No. 41/16, 2004.
171. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 53, 2005.
172. *Compendium of food additive specifications: addendum 12.* FAO Food and Nutrition Paper, No. 52, Add. 12, 2004.
173. *Evaluation of certain food additives* (Sixty-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 928, 2005.
174. *Safety evaluation of certain food additives*. WHO Food Additives Series, No 54, 2005.
175. *Compendium of food additive specifications*: *addendum 13.* FAO Food and Nutrition Paper, No. 52, Add. 13 (with Errata), 2005.
176. *Evaluation of certain food contaminants* (Sixty-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 930, 2005.
177. *Safety evaluation of certain contaminants in food*. WHO Food Additives Series, No. 55/FAO Food and Nutrition Paper, No. 82, 2006.
178. *Evaluation of certain food additives* (Sixty-fifth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 934, 2006.
179. *Safety evaluation of certain food additives.* WHO Food Additives Series, No. 56, 2006.

180. *Combined compendium of food additive specifications.* FAO JECFA Monographs 1, Volumes 1–4, 2005, 2006.
181. *Evaluation of certain veterinary drug residues in food* (Sixty-sixth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 939, 2006.
182. *Residue evaluation of certain veterinary drugs.* FAO JECFA Monographs 2, 2006.
183. *Toxicological evaluation of certain veterinary drug residues in food*. WHO Food Additives Series, No. 57, 2006.
184. *Evaluation of certain food additives and contaminants* (Sixty-seventh report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 940, 2007.
185. *Compendium of food additive specifications.* FAO JECFA Monographs 3, 2006.
186. *Safety evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 58, 2007.
187. *Evaluation of certain food additives and contaminants* (Sixty-eighth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 947, 2007.
188. *Safety evaluation of certain food additives and contaminants*. WHO Food Additives Series, No. 59, 2008.
189. *Compendium of food additive specifications*, FAO JECFA Monographs 4, 2007.
190. *Evaluation of certain food additives* (Sixty-ninth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 952, 2009.
191. *Safety evaluation of certain food additives*. WHO Food Additives Series, No. 60, 2009.
192. *Compendium of food additive specifications*. FAO JECFA Monographs 5, 2009.
193. *Evaluation of certain veterinary drug residues in food* (Seventieth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 954, 2009.
194. *Toxicological evaluation of certain veterinary drug residues in food.* WHO Food Additives Series, No. 61, 2009.
195. *Residue evaluation of certain veterinary drugs*. FAO JECFA Monographs 6, 2009.
196. *Evaluation of certain food additives* (Seventy-first report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 956, 2010.
197. *Safety evaluation of certain food additives.* WHO Food Additives Series, No. 62, 2010.
198. *Compendium of food additive specifications.* FAO JECFA Monographs 7, 2009.
199. *Evaluation of certain contaminants in food* (Seventy-second report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 959, 2011.
200. *Safety evaluation of certain contaminants in food.* WHO Food Additives Series, No. 63/FAO JECFA Monographs 8, 2011.

201. *Residue evaluation of certain veterinary drugs*. FAO JECFA Monographs 9, 2010.
202. *Evaluation of certain food additives and contaminants* (Seventy-third report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 960, 2011.
203. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 64, 2011.
204. *Compendium of food additive specifications.* FAO JECFA Monographs 10, 2010.
205. *Evaluation of certain food additives and contaminants* (Seventy-fourth report of the Joint FAO/WHO Expert Committee on Food Additives). WHO Technical Report Series, No. 966, 2011.
206. *Safety evaluation of certain food additives and contaminants.* WHO Food Additives Series, No. 65, 2012.
207. *Compendium of food additive specifications.* FAO JECFA Monographs 11, 2011.

ANNEX 2

ABBREVIATIONS USED IN THE MONOGRAPHS

2-AAF	2-acetylaminofluorene
4-AP	4-aminopyridine
4-HDA	4-hydroxyalkenal
8-OH-dG	8-hydroxy-2′-deoxyguanosine
ADHD	attention deficit hyperactivity disorder
ADI	acceptable daily intake
ADRA2A	adrenergic receptor alpha 2A
AFB	aflatoxin B (e.g. AFB_1)
AIC	Akaike's information criterion
AIDS	acquired immunodeficiency syndrome
ALMA	Aluminium-Maladie d'Alzheimer
ALT	alanine aminotransferase
AP	alkaline phosphatase
ApoE	apolipoprotein E
ARfD	acute reference dose
AST	aspartate aminotransferase
AT	atopy
ATPase	adenosine triphosphatase
AUC	area under the concentration versus time curve
BEA	beauvericin
BMC	bone mineral content
BMD	benchmark dose; bone mineral density
BMDL	lower limit of the 95% confidence interval on the benchmark dose
BMR	benchmark response
Bt	*Bacillus thuringiensis*
bw	body weight
CAS	Chemical Abstracts Service
CCCF	Codex Committee on Contaminants in Foods
CCFA	Codex Committee on Food Additives
CI	confidence interval
CIT	citrinin
C_{max}	maximum concentration
COMT	catechol *O*-methyl-transferase
ConA	concanavalin A
CPK	creatine phosphokinase
CYP	cytochrome P450
DAT1	dopamine transporter
DNA	deoxyribonucleic acid
DON	deoxynivalenol
DRD4	dopamine D4 receptor
EC	Enzyme Commission
EFSA	European Food Safety Authority

ELEM	equine leukoencephalomalacia
ELISA	enzyme-linked immunosorbent assay
ESI	electrospray ionization
EU	European Union
F	female; filial generation
FAO	Food and Agriculture Organization of the United Nations
FB	fumonisin B (e.g. FB_1, FB_2, FB_3)
FB_T	total fumonisins
FC	fumonisin C
FFQ	food frequency questionnaire
FSANZ	Food Standards Australia New Zealand
GD	gestation day
GEMS/Food	Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme
GGT	γ-glutamyl transferase
GHA	global hyperactivity aggregate
GLC	gas–liquid chromatography
GLP	good laboratory practice
GMP	good manufacturing practice
GSFA	Codex General Standard for Food Additives
GSH	glutathione (reduced)
GST	glutathione *S*-transferase
GSTP	placental glutathione *S*-transferase
HA	hyperactivity
HACCP	hazard analysis and critical control point
HFB	totally hydrolysed fumonisin B
HIV	human immunodeficiency virus
HNMT	histamine *N*-methyl-transferase
HPLC	high-performance liquid chromatography
HTLV	human T-cell lymphotropic virus
IARC	International Agency for Research on Cancer
IC_{50}	median inhibitory concentration
ICGMA	International Council of Grocery Manufacturer Associations
ICP	inductively coupled plasma
ID_{50}	median inhibitory dose
IFN	interferon (e.g. IFN-γ)
IG_{50}	median growth inhibitory concentration
Ig	immunoglobulin (e.g. IgA, IgE, IgG, IgM)
IL	interleukin (e.g. IL-1β, IL-2)
INCA-2	Second National Individual Survey on Food Consumption (France)
INS	International Numbering System
IPCS	International Programme on Chemical Safety
JECFA	Joint FAO/WHO Expert Committee on Food Additives
LA	linoleic acid
LC	liquid chromatography
LD_{50}	median lethal dose
LDH	lactate dehydrogenase

LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOED	lowest-observed-effect dose
LOEL	lowest-observed-effect level
LOQ	limit of quantification
LPS	lipopolysaccharide
M	male
MDA	malondialdehyde
ML	maximum level
MMSE	Mini-Mental State Examination
MON	moniliformin
MS	mass spectrometry
MS/MS	tandem mass spectrometry
MTD	maximum tolerated dose
MTT	3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide
NFT	neurofibrillary tangles
NIV	nivalenol
NMR	nuclear magnetic resonance
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level
NS	not specified
NTD	neural tube defect
NTP	United States National Toxicology Program
OA	oleic acid
OECD	Organisation for Economic Co-operation and Development
OES	optical emission spectrometry
OR	odds ratio
OTA	ochratoxin A
P	probability
PA	penicillic acid; palmitic acid
PAQUID	Personnes Âgées Quid
PCR	polymerase chain reaction
PH	partial hepatectomy
PHA	phytohaemagglutinin
PHA-P	phytohaemagglutinin P
PHFB	partially hydrolysed fumonisin B
PMTDI	provisional maximum tolerable daily intake
PND	postnatal day
PPARα	peroxisome proliferator-activated receptor alpha
PPE	porcine pulmonary oedema
PRRSV	porcine reproductive and respiratory syndrome virus
p-Si	poly-silicon
PST-M	phenolsulfotransferase-M
PST-P	phenolsulfotransferase-P
PTWI	provisional tolerable weekly intake
RNA	ribonucleic acid
RR	relative risk

RSD	relative standard deviation
RSD_r	relative standard deviation for within-laboratory repeatability
RSD_R	relative standard deviation for between-laboratory reproducibility
RT-PCR	reverse transcriptase polymerase chain reaction
S1P	sphingoid base 1-phosphate
S1PR	sphingoid base 1-phosphate receptor
S9	9000 × *g* rat liver supernatant
SAR	Special Administrative Region
SCOOP	Scientific Cooperation
SD	standard deviation
SDS	sodium dodecyl sulfate
SEM	standard error of the mean
SOD	superoxide dismutase
SPE	solid-phase extraction
T_3	triiodothyronine
T_4	thyroxine
TBARS	thiobarbituric acid reactive substances
TCA	tricarballylic acid
TDI	tolerable daily intake
TLC	thin-layer chromatography
TNFα	tumour necrosis factor alpha
TOS	total organic solids
TUNEL	terminal deoxynucleotidyl transferase–mediated deoxyuridine diphosphate nick-end labelling
UGT	uridine diphosphate glucuronosyltransferase
UNESDA	Union of European Soft Drinks Associations
UPLC	ultra high-performance liquid chromatography
USA	United States of America
USDA	United States Department of Agriculture
USFDA	United States Food and Drug Administration
UV	ultraviolet
WHO	World Health Organization
w/w	weight per weight
ZEA	zearalenone

ANNEX 3

JOINT FAO/WHO EXPERT COMMITTEE ON FOOD ADDITIVES

Rome, 14–23 June 2011

MEMBERS

Dr M. Bolger, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA

Professor M.C. de Figueiredo Toledo, Faculdade de Engenharia de Alimentos, Universidade Estadual de Campinas, Campinas, Brazil

Dr M. DiNovi, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA

Dr Y. Kawamura, Division of Food Additives, National Institute of Health Sciences, Tokyo, Japan

Dr A. Mattia, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*Vice-Chairperson*)

Mrs I. Meyland, National Food Institute, Technical University of Denmark, Søborg, Denmark (*Chairperson*)

Dr Z. Olempska-Beer, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA

Professor A. Renwick, Emeritus Professor, School of Medicine, University of Southampton, Ulverston, England (*Joint Rapporteur*)

Dr S. Resnik, Facultad de Ciencias Exactas y Naturales, Ciudad Universitaria, Buenos Aires, Argentina

Dr J. Schlatter, Nutritional and Toxicological Risks Section, Federal Office of Public Health, Zurich, Switzerland

Ms E. Vavasour, Ottawa, Ontario, Canada

Dr M. Veerabhadra Rao, Department of the President's Affairs, Al Ain, United Arab Emirates

Professor R. Walker, Ash, Aldershot, Hantfordshire, England

Mrs H. Wallin, Finnish Food Safety Authority (Evira), Helsinki, Finland (*Joint Rapporteur*)

SECRETARIAT

Dr A. Agudo, Catalan Institute of Oncology, L'Hospitalet de Llobregat, Spain (*WHO Temporary Adviser*)

Mr D. Arcella, European Food Safety Authority, Parma, Italy (*FAO Expert*)

Dr D. Benford, Food Standards Agency, London, England (*WHO Temporary Adviser*)

Mrs G. Brisco, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Codex Secretariat*)

Dr A. Bruno, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Codex Secretariat*)

Ms A. Bulder, Centre for Substances and Integrated Risk Assessment, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (*WHO Temporary Adviser*)

Mrs V. Carolissen-Mackay, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Codex Secretariat*)

Dr C. Carrington, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*WHO Temporary Adviser*)

Dr R. Danam, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*WHO Temporary Adviser*)

Dr J.A. Edgar, Honorary Fellow, CSIRO Food and Nutritional Sciences, North Ryde, Australia (*FAO Expert*)

Mr M. Feeley, Food Directorate, Health Canada, Ottawa, Ontario, Canada (*WHO Temporary Adviser*)

Dr D. Folmer, Center for Food Safety and Applied Nutrition, Food and Drug Administration, College Park, MD, USA (*FAO Expert*)

Ms T. Hambridge, Food Standards Australia New Zealand, Canberra, Australia (*WHO Temporary Adviser*)

Dr H. Kim, Joint FAO/WHO Food Standards Programme, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Codex Secretariat*)

Dr K. Kpodo, Food Chemistry Division, CSIR-Food Research Institute, Accra, Ghana (*FAO Expert*)

Dr J.-C. Leblanc, Food Risk Assessment Division, Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail (ANSES), Maisons-Alfort, France (*FAO Expert*)

Professor S.M. Mahungu, Department of Dairy, Food Science and Technology, Egerton University, Egerton, Kenya (*FAO Expert*)

Dr U.W. Mueller, Food Standards Australia New Zealand, Canberra, Australia (*WHO Temporary Adviser*)

Professor S. Rath, Department of Analytical Chemistry, University of Campinas, Campinas, São Paulo, Brazil (*FAO Expert*)

Dr R.T. Riley, Agricultural Research Service, United States Department of Agriculture, Athens, GA, USA (*WHO Temporary Adviser*)

Ms M. Sheffer, Ottawa, Ontario, Canada (*WHO Editor*)

Dr A. Tritscher, Department of Food Safety and Zoonoses, World Health Organization, Geneva, Switzerland (*WHO Joint Secretary*)

Dr T. Umemura, Biological Safety Research Center, National Institute of Health Sciences, Ministry of Health, Labour and Welfare, Tokyo, Japan (*WHO Temporary Adviser*)

Dr A. Wennberg, Nutrition and Consumer Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Joint Secretary*)

Dr G. Wolterink, Centre for Substances and Integrated Risk Assessment, National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands (*WHO Temporary Adviser*)

Dr F. Wu, Department of Environmental and Occupational Health, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, PA, USA (*WHO Temporary Adviser*)

ANNEX 4

TOLERABLE AND ACCEPTABLE INTAKES, OTHER TOXICOLOGICAL INFORMATION AND INFORMATION ON SPECIFICATIONS

Food additives evaluated toxicologically or assessed for dietary exposure

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
Aluminium-containing food additives (including new food additives potassium aluminium silicate and potassium aluminium silicate–based pearlescent pigments)	N, T[b]	**The Committee established a provisional tolerable weekly intake (PTWI) of 2 mg/kg body weight** based on a no-observed-adverse-effect level (NOAEL) of 30 mg/kg body weight per day and application of an uncertainty factor of 100. The PTWI applies to all aluminium compounds in food, including food additives. **The previous PTWI of 1 mg/kg body weight was withdrawn**. For adults, the estimates of mean dietary exposure to aluminium-containing food additives from consumption of cereals and cereal-based products are up to the PTWI. Estimates of dietary exposure of children to aluminium-containing food additives, including high dietary exposures (e.g. 90th or 95th percentile), can exceed the PTWI by up to 2-fold. For potassium aluminium silicate–based pearlescent pigments at the maximum proposed use levels and using conservative estimates, anticipated dietary exposure at the highest range of estimates is 200 times higher than the PTWI. The Committee emphasized that whereas substances that have long half-lives and accumulate in the body are not generally considered suitable for use as food additives, consumption of aluminium-containing food additives would not be a health concern, provided that total dietary exposure to aluminium is below the PTWI. The Committee recommended that provisions for food additives containing aluminium included in the Codex General Standard for Food Additives should be compatible with the revised PTWI for aluminium compounds of 2 mg/kg body weight as aluminium from all sources.

Annex 4 (contd)

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
Benzoe Tonkinensis	N, T	**The Committee concluded that the available data were inadequate to establish an acceptable daily intake (ADI)** because of the variability in composition of Benzoe Tonkinensis and the inadequate characterization of the material tested. The margin of exposure between the conservative dietary exposure estimate of 0.2 mg/kg body weight per day and the NOAEL of 500 mg/kg body weight per day identified in a 90-day oral toxicity study in rats is 2500. Given this margin of exposure as well as the nature of the hepatic effects observed at doses above the NOAEL and the negative genotoxicity results, **the Committee concluded that Benzoe Tonkinensis would not pose a health concern at current estimated dietary exposures, provided that it complies with the tentative specifications prepared at the current meeting, when used as a flavouring agent and in accordance with good manufacturing practice.** The Committee also noted that exposure to benzoic acid and benzyl benzoate from the use of Benzoe Tonkinensis is well below the upper limit of the group ADI (0–5 mg/kg body weight) for benzyl derivatives, and exposure to vanillin is also well below the upper limit of its ADI (0–10 mg/kg body weight). The Committee further noted that benzoic acid, one of the major components of Benzoe Tonkinensis, is used as a preservative, but that Benzoe Tonkinensis has not been assessed for this use.
Glycerol ester of gum rosin (GEGR)	R, T	**The Committee withdrew the group ADI for GEGR and glycerol ester of wood rosin (GEWR) and established a temporary group ADI for GEGR and GEWR of 0–12.5 mg/kg body weight,** pending the submission of the full reports of the 90-day toxicity studies on GEGR as well as additional compositional information on the GEWR from *Pinus elliottii*. The Committee noted that the temporary group ADI will be withdrawn if the requested information is not submitted by the end of 2012.
Glycerol ester of tall oil rosin (GETOR)	R, T	**The Committee was unable to complete the evaluation of GETOR** because additional data are required to characterize the GETOR in commerce. Validated methods for the determination of the substances considered in the specifications are also required. The above information should be submitted by the end of 2012.

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
Glycerol ester of wood rosin (GEWR)	R, T	**The Committee withdrew the group ADI for GEGR and GEWR and established a temporary group ADI for GEGR and GEWR of 0–12.5 mg/kg body weight,** applying an additional uncertainty factor of 2, because new information raises questions about the identity and composition of the product in commerce. Additional compositional information on the GEWR from *Pinus elliottii* to assess similarity with the GEWR from *Pinus palustris* is required. The Committee noted that the temporary group ADI will be withdrawn if the requested information is not submitted by the end of 2012.
Octenyl succinic acid (OSA) modified gum arabic	R	**The Committee deferred further evaluation of OSA modified gum arabic** pending the submission of data on its stability in food and on the extent to which it is hydrolysed in the gastrointestinal tract, to be provided by the end of 2013. The existing temporary ADI "not specified"[c] was retained.
Polydimethyl siloxane	M	**The Committee withdrew the temporary ADI of 0–0.8 mg/kg body weight and re-established the ADI of 0–1.5 mg/kg body weight,** originally established at the eighteenth meeting.
Ponceau 4R	R	**The Committee concluded that new data do not indicate a need to revise the existing ADI of 0–4 mg/kg body weight** and that dietary exposure to Ponceau 4R does not present a health concern.
Pullulan	R	Dietary exposure to pullulan as a dietary fibre could reach 1 g/kg body weight per day for children (2–5 years old) and 0.4 g/kg body weight per day for the general population (2 years of age and older). These estimates are 8 and 20 times lower, respectively, than the no-observed-effect level (NOEL) observed in the 90-day rat study evaluated previously. Gastrointestinal effects observed in humans should be taken into account when considering appropriate use levels. The Committee stressed that it assessed the safety of use and not the efficacy of pullulan used as a dietary fibre. **The Committee maintained the previously established ADI "not specified"[c] for the previously evaluated food additive uses.**
Pullulanase from *Bacillus deramificans* expressed in *Bacillus licheniformis*	N	**The Committee established an ADI "not specified"[c] for pullulanase from *B. deramificans* expressed in *B. licheniformis*** when used in the applications specified and in accordance with good manufacturing practice.

Annex 4 (contd)

Food additive	Specifications[a]	Acceptable or tolerable daily intakes and other toxicological recommendations
Quinoline Yellow	R, T	**The Committee established a temporary ADI of 0–5 mg/kg body weight,** incorporating an additional 2-fold uncertainty factor, pending submission of requested toxicological studies by the end of 2013. **The previously established ADI of 0–10 mg/kg body weight was withdrawn.** The conservative exposure estimates were within the range of the temporary ADI. Additional information on the composition of the product in commerce is required, in particular relating to the identity and purity of the unmethylated form of Quinoline Yellow.
Sunset Yellow FCF	M	**The Committee established an ADI of 0–4 mg/kg body weight and withdrew the previous ADI of 0–2.5 mg/kg body weight**. The Committee concluded that dietary exposure to Sunset Yellow FCF does not present a health concern.

[a] M, existing specifications maintained; N, new specifications prepared; R, existing specifications revised; T, tentative specifications.

[b] For potassium aluminium silicate and pearlescent pigments containing potassium aluminium silicate.

[c] ADI "not specified" is used to refer to a food substance of very low toxicity that, on the basis of the available data (chemical, biochemical, toxicological and other) and the total dietary exposure to the substance arising from its use at the levels necessary to achieve the desired effects and from its acceptable background levels in food, does not, in the opinion of the Committee, represent a hazard to health. For that reason, and for the reasons stated in the individual evaluations, the establishment of an ADI expressed in numerical form is not deemed necessary. An additive meeting this criterion must be used within the bounds of good manufacturing practice—i.e. it should be technologically efficacious and should be used at the lowest level necessary to achieve this effect, it should not conceal food of inferior quality or adulterated food, and it should not create a nutritional imbalance.

Food additives considered for specifications only

Food additive	Specifications[a]
β-Apo-8′-carotenal	R
β-Apo-8′-carotenoic acid ethyl ester	R
β-Carotene, synthetic	R
Hydroxypropyl methyl cellulose	R[b]
Magnesium silicate, synthetic	R
Modified starches	R
Nitrous oxide	R
Sodium carboxymethyl cellulose	R
Sucrose monoesters of lauric, palmitic or stearic acid	R

[a] R, existing specifications revised.

[b] The Committee concluded that levels of propylene chlorohydrins up to the new limit of not more than 1 mg/kg for the sum of both isomers in hydroxypropyl methyl cellulose were not of toxicological concern.

Analytical methods for food additives in the Combined Compendium of Food Additive Specifications, Volume 4 (FAO JECFA Monographs 1, 2006)

Food additive	Method[a]
Colouring matters content by spectrophotometry	R, T

[a] R, existing method revised; T, tentative method.

Contaminants evaluated toxicologically

Cyanogenic glycosides

The Third Session of the Codex Committee on Contaminants in Food (CCCF) in 2009 requested that JECFA reconsider the available data on cyanogenic glycosides, advise on the public health implications of cyanogenic glycosides and their derivatives in food and decide whether risk assessment is feasible and appropriate.

Reports of acute human poisoning associated with the consumption of foods containing cyanogenic glycosides were reviewed. The Committee therefore considered it appropriate to establish an acute reference dose (ARfD) for cyanogenic glycosides, expressed as cyanide equivalents. In addition, as there are a number of human diseases, specifically konzo, tropical ataxic neuropathy and iodine deficiency disorders, associated with the chronic consumption of underprocessed cassava as a staple food, it was recognized that the derivation of a chronic health-based guidance value would also be relevant.

Derivation of the ARfD

Following review of a developmental toxicity study with linamarin, the Committee considered this study as suitable for establishing an ARfD. Benchmark dose (BMD) modelling of the data from this study provided a lower limit on the benchmark dose for a 10% response ($BMDL_{10}$) for linamarin of 85 mg/kg body weight for increased skeletal defects in developing hamster fetuses following acute exposure of maternal animals. Although the study did not use dietary exposure, gavage dosing was considered relevant to establishing the ARfD.

Following application of a 100-fold uncertainty factor, the Committee established an ARfD for linamarin of 0.9 mg/kg body weight (equivalent to 0.09 mg/kg body weight as cyanide). This value was considered, when compared on a cyanide molar basis, to also be applicable to other cyanogenic glycosides. Therefore, the Committee recommended conversion of the ARfD for linamarin to a cyanide-equivalent dose of 0.09 mg/kg body weight. This cyanide-equivalent ARfD applies only to foods containing cyanogenic glycosides as the main source of cyanide.

Derivation of the provisional maximum tolerable daily intake (PMTDI)

In a 13-week United States National Toxicology Program study not previously evaluated by the Committee, in which exposure to sodium cyanide was continuous via drinking-water, a variety of effects related to male reproductive organs were observed—namely, decreased cauda epididymis weights, decreased testis weights and decreased testicular spermatid concentration. Dose–response analysis of continuous data on absolute cauda epididymis weights generated the lowest BMDL for a one standard deviation response ($BMDL_{1SD}$) of 1.9 mg/kg body weight per day. On the basis of this $BMDL_{1SD}$, the Committee established a PMTDI of 0.02 mg/kg body weight by applying a 100-fold uncertainty factor. The Committee decided that it was not necessary to apply an additional uncertainty factor to account for the absence of a long-term study, considering the generally acute nature of cyanide toxicity and the sensitivity of the effect (i.e. the reduction of absolute cauda epididymis weight).

Comparison of estimated dietary exposures with health-based guidance values and the impact of maximum limits (MLs) on dietary exposure

Estimated dietary exposures to total available hydrocyanic acid (HCN) were converted to cyanide equivalents and compared with the health-based guidance values established by the Committee at this meeting.

From the national acute dietary exposure estimates available to the Committee for review, the ARfD of 0.09 mg/kg body weight as cyanide equivalents was exceeded 3-fold for cassava for adults (based on raw samples), less than 2-fold for apple juice for children, between 2- and 5-fold for bitter apricot kernels and up to 10-fold for ready-to-eat cassava chips/crisps, depending on the population group. If ready-to-eat cassava chips contained a level equivalent to the recently established ML in Australia and New Zealand of 10 mg/kg as HCN, there was only a marginal exceedance of the ARfD for children. These results are based on dietary exposure to total HCN, which represents the maximum possible exposure for foods containing cyanogenic glycosides.

Based on national estimates of chronic dietary exposure to total HCN, there is also the potential to exceed the PMTDI of 0.02 mg/kg body weight as cyanide for populations reliant on cassava as a staple food: between 1- and 3-fold for children and between 1- and 2-fold for adults. There is also a potential for those populations not reliant on cassava to exceed the PMTDI: between 1- and 5-fold for children and between 1- and 3-fold for adults. For Australia and New Zealand, ready-to-eat cassava chips were the major contributor to dietary exposure to HCN (84–93%). When the cassava chips contain a level equivalent to the ML of 10 mg/kg as HCN, all mean dietary exposures were below the PMTDI. High-percentile exposures for children were between 1- and 2-fold above the PMTDI. All chronic dietary exposure estimates based on exposures from flavouring agents did not exceed the PMTDI. These results are based on dietary exposure to total HCN, which is a worst-case scenario.

Application of the ML of 50 mg/kg as HCN for sweet cassava could result in dietary exposures that exceed the ARfD by less than 2-fold for the general population and up to 4-fold for children and exceed the PMTDI by between 2- and 10-fold, depending on the population group assessed. These estimates do not take into consideration any reduction in concentration of total HCN as a result of food preparation or processing. For the ML of 10 mg/kg as HCN for cassava flour, there are no estimates of dietary exposure available that exceed the ARfD or PMTDI. This is supported by the maximum amount of food that can be consumed based on existing Codex MLs before the health-based guidance values would be exceeded, which is as low as 25 g/day for cassava for chronic exposure. More detailed estimates of cassava and cassava flour consumption and concentrations in food for cassava-eating communities would help in supporting the conclusion that dietary exposures to total HCN could exceed health-based guidance values.

The ML for sweet cassava is for the raw product. If the starting level of HCN in the raw sweet cassava were 50 mg/kg as HCN, the minimum effective processing would result in a concentration of 15 mg/kg as HCN, and the most effective processing would give an HCN concentration of 2 mg/kg.

ARfD: 0.09 mg/kg body weight as cyanide (applies only to foods containing cyanogenic glycosides as the main source of cyanide)

PMTDI: 0.02 mg/kg body weight as cyanide

Fumonisins

For the current evaluation of fumonisins, the Committee reviewed all relevant studies performed on fumonisins since 2001.

Exposure to fumonisins has been associated with a wide range of effects, which are often species and sex specific. Laboratory studies have identified the liver as the most sensitive organ in mice and the kidney as the most sensitive organ in rats.

Studies suitable for dose–response analysis have been conducted with rodents either employing purified fumonisin B_1 (FB_1) or using *Fusarium verticillioides* culture material containing FB_1. The latter studies typically use FB_1 as

a marker for dietary exposure to the fumonisins and other metabolites of *Fusarium*. The studies employing purified FB_1 are generally better in experimental design for dose–response analysis. However, the Committee concluded that the studies with culture material were of sufficient quality to clearly indicate that other toxins produced by *F. verticillioides* either add to or potentiate the toxicity of FB_1. Although naturally contaminated corn would probably be more representative of actual human dietary exposure than either purified FB_1 or culture material, no suitable studies were identified that used naturally contaminated corn as a test material. As the implications are somewhat different, the Committee evaluated studies with purified FB_1 and *F. verticillioides* culture material separately.

For pure FB_1, the lowest identified $BMDL_{10}$ was 165 µg/kg body weight per day for megalocytic hepatocytes in male mice. Using an uncertainty factor of 100 for intraspecies and interspecies variation, the Committee derived a PMTDI of 2 µg/kg body weight per day. As this was the same value as the previously established group PMTDI for FB_1, FB_2 and FB_3, alone or in combination, this group PMTDI was retained.

For culture material, the lowest identified $BMDL_{10}$ using FB_1 as a marker was 17 µg/kg body weight per day for renal toxicity in male rats. The Committee chose not to establish a health-based guidance value for culture material, because its composition was not well characterized and may not be representative of natural contamination.

The Committee concluded that, based on the national and international estimates, dietary exposure to FB_1 for the general population ranges from 0.12×10^{-3} to 7.6 µg/kg body weight per day at the mean, whereas the 95th percentile exposure was estimated to be up to 33.3 µg/kg body weight per day. Dietary exposure to total fumonisins for the general population would range, for a consumer with average consumption, from 0.087×10^{-3} to 14.4 µg/kg body weight per day, whereas for consumers with high consumption, exposure would be up to 44.8 µg/kg body weight per day. Maize is still the predominant source of exposure to FB_1 and total fumonisins.

Comparison of these estimates with the group PMTDI indicates that the group PMTDI is exceeded at the population level in some regions within some countries. The Committee concluded that adverse effects from fumonisin exposure may occur and that reduction of exposure to fumonisin and other toxins produced by *F. verticillioides* is highly desirable, particularly in areas of the world where maize is a major dietary staple food and where high contamination can occur.

As fumonisins do not carry over from feed to animal products in significant amounts, the occurrence of fumonisins in feed was considered not to be a human health concern.

The Committee concluded that implementation of the MLs proposed by CCCF could significantly reduce exposure (by more than 20%) to total fumonisins in six Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption clusters (A, B, D, F, G, K). The main contribution to reduction was due to the proposed Codex ML for the category "Corn/maize grain, unprocessed". The Committee noted that implementation

of the proposed MLs would result in rejection of 1–88% of "Corn/maize grain, unprocessed" and 4–57% of "Corn/maize flour/meal" across the clusters. The Committee also noted that the national estimates of exposure to fumonisins show that the exceedance of the PMTDI occurs only in limited regions presenting high maize consumption levels and highly contaminated maize.

The Committee concluded that no or little effect was noticed on the international exposure estimates resulting from the implementation of MLs higher than those proposed by CCCF.

Group PMTDI for FB_1, FB_2 and FB_3, alone or in combination, of 2 µg/kg body weight was retained

This volume contains monographs prepared at the seventy-fourth meeting of the Joint FAO/WHO Expert Committee on Food Additives (JECFA), which met in Rome, Italy, from 14 to 23 June 2011.

The toxicological monographs in this volume summarize the safety data on a number of food additives: aluminium-containing food additives, Benzoe Tonkinensis, Ponceau 4R, pullulanase from *Bacillus deramificans* expressed in *Bacillus licheniformis*, Quinoline Yellow and Sunset Yellow FCF.

This volume also contains monographs summarizing the toxicological and dietary exposure data for the contaminants cyanogenic glycosides and fumonisins.

This volume and others in the WHO Food Additives Series contain information that is useful to those who produce and use food additives and veterinary drugs and those involved with controlling contaminants in food, government and food regulatory officers, industrial testing laboratories, toxicological laboratories and universities.

ISBN 978 92 4 166065 5