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EVALUATION OF CERTAIN CONTAMINANTS IN FOOD

Seventy-second report of the
Joint FAO/WHO Expert Committee on
Food Additives

Food and Agriculture Organization of the United Nations

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Rome, 16–25 February 2010

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Joint monographs containing summaries of relevant analytical and technical data and toxicological evaluations are available from WHO under the title:

Safety evaluation of certain contaminants in food. WHO Food Additives Series, No. 63

and from FAO under the title:

Safety evaluation of certain contaminants in food. FAO JECFA Monographs 8

1. Introduction

The Joint FAO/WHO Expert Committee on Food Additives (JECFA) met in Rome, Italy, from 16 to 25 February 2010. The meeting, which was dedicated to the evaluation of certain contaminants in food, was opened by Dr Ezzeddine Boutrif, Director of the Nutrition and Consumer Protection Division of the Agriculture and Consumer Protection Department of the Food and Agriculture Organization of the United Nations (FAO), on behalf of the Directors General of FAO and the World Health Organization (WHO). Dr Boutrif noted that the work performed by JECFA in the area of the risk assessment of chemicals in food is a cornerstone in the process of providing international guidance to ensure that food safety and food quality measures are based on science. He emphasized that this work remains an important and high priority for FAO and WHO. Dr Boutrif also noted the increased importance that was placed on food security and the right to food by the international community at the recent World Summit on Food Security, held in November 2009. He suggested that a scarce food supply may increase exposure to contaminants in food and stressed that efforts to increase food production should take into consideration factors aiming to reduce food contamination as far as possible.

FAO and WHO recently concluded the joint project to update the principles and methods for the risk assessment of chemicals in food, and Dr Boutrif thanked all those who had contributed to this major accomplishment.

1.1 Declarations of interests

The Secretariat informed the Committee that all experts participating in the seventy-second meeting had completed declaration of interest forms and that no conflicts had been identified. The following declared interest and potential conflict was discussed by the Committee: Dr Leila Barraj participated in an industry-sponsored assessment of acrylamide intake and acrylamide adduct levels based on publicly available data and therefore abstained from discussions on this compound.

2. General considerations

As a result of the recommendations of the first Joint FAO/WHO Conference on Food Additives, held in September 1955 (*1*), there have been 71 previous meetings of the Committee (Annex 1). The present meeting was convened on the basis of a recommendation made at the seventy-first meeting (Annex 1, reference *196*).

The tasks before the Committee were:

- to elaborate further principles for evaluating the safety of contaminants in food (section 2);
- to undertake toxicological evaluations of certain contaminants in food (section 3 and Annex 2).

2.1 Report from the Third Session of the Codex Committee on Contaminants in Foods (CCCF)

The Chair of the Codex Committee on Contaminants in Foods (CCCF), Dr Martijn Weijtens, Ministry of Agriculture, Nature and Food Quality, Netherlands, reported on the outcome of the Third Session of the CCCF and highlighted the importance of the work of JECFA for the development of international food safety standards in the framework of the Codex Alimentarius Commission. He underlined the necessity to particularly consider animal feed contaminants as potential hazards for human health. He also stressed the relevance of good communication between CCCF and JECFA on requests for evaluations of contaminants in food and on the possible impact of the outcomes of the evaluations. The importance of collecting and submitting representative data on the occurrence of contaminants in food from a variety of sources and geographical areas for evaluation by JECFA was also discussed, and Dr Weijtens indicated that the CCCF Secretariat would strongly support the plan by FAO and WHO to hold a workshop on the matter during the next session of CCCF, to be held in Izmir, Turkey, on 25–29 April 2010.

2.2 Modelling of dose–response data

The present meeting used dose–response modelling to evaluate exposure-related effects and to derive a point of departure (POD) for the estimation of a margin of exposure (MOE) or health-based guidance value. The method used was based on that employed at the sixty-fourth meeting of the Committee (Annex 1, reference *176*). At the present meeting, the Committee proposed and followed the steps given below:

- The data are assessed for exposure-related responses.
- The biological relevance to human health of responses found in animal studies is assessed.
- In assessment of the data from epidemiological studies, it may be necessary to make adjustments to the data that involve both the dose (e.g. to take other sources of exposure into account) and the outcome (e.g. conversion of risk per person-year to risk per person over a lifetime).
- A benchmark response (BMR) for the effects to be modelled is selected. The sixty-fourth meeting of the Committee selected a BMR of 10% for carcinogenicity data from 2-year studies in rodents, but other BMRs may be more appropriate for epidemiological studies with large numbers of subjects, for other quantal end-points or for continuous data.
- The mathematical models appropriate for the chosen end-points (continuous or quantal data) are selected.
- The models are fitted to the selected data using suitable software (the United States Environmental Protection Agency's [USEPA] benchmark dose [BMD] software [BMDS] and RIVM's PROAST have been used by the Committee in its evaluations).
- Results from the models that provide acceptable fits are used for derivation of the POD (e.g. in section 3.4 of this report, when the BMDS was used for furan, a *P*-value of >0.1 for the goodness of fit was used to define an acceptable fit). At both the sixty-fourth meeting and the present meeting, the lowest lower confidence limit on the benchmark dose (BMDL) from the accepted models was used, except when data from a more robust or better-designed study measuring the same response resulted in less uncertainty and a slightly higher BMDL (see section 3.4 of this report for an example of this).

In the report, the BMR(s) and software used are stated, and the effects selected for modelling and the ranges of BMDs and BMDLs estimated by the different acceptable fits are tabulated.

In the monograph, the output of the models is given in tabular and graphical forms. The table of results shows the model, the *P*-value of the goodness of fit test, the BMD and the BMDL. Ideally, the graph should show results for the model resulting in the lowest BMDL, the dose–response data with the fitted curve and the confidence intervals at different dose levels and should indicate the position of the BMD; the graph should also show the curve for the lower bound on the BMD and indicate the position of the BMDL (illustrative examples using BMDS are shown below).

The Committee recognized that use of the lowest BMDL from the accepted models could result in a POD from a less robust data set being used in preference to the BMDL from a better data set that showed a better fit and higher BMDL in the presence of a comparable BMD. The Committee was aware of developments in combining the outputs of different models to generate an average model, the output of which includes all models weighted according to their goodness of fit (*2*).

The Committee recognized that the use of dose–response modelling is a developing field and recommends to the Joint FAO/WHO Secretariat that an expert working group be established to review progress and develop detailed guidance for the application of the methods most suitable to the work of the Committee. The working group should, inter alia, address the following aspects:

- the use of constraints when modelling;
- the weighting of model outcomes and model averaging;
- goodness of fit criteria;
- how human data might be used for dose–response modelling to derive a POD;
- presentation of modelling outcomes in JECFA publications.

Example of data tabulation for the monograph

The example chosen for illustrative purposes (Table 1) is the modelling output for hepatocellular adenoma and carcinoma for female mice treated with furan (see section 3.4 of this report for details).

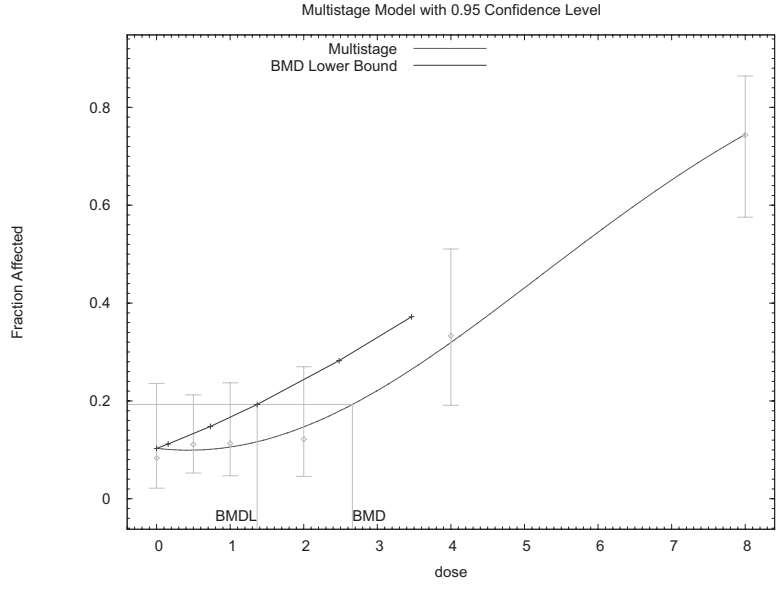
Table 1
Example of modelling output for hepatocellular adenoma and carcinoma for female mice treated with furan

	Gamma	Logistic	LogLogistic	LogProbit	Multistage	Multistage-Cancer	Probit	Weibull	Quantal-Linear
AIC	235.33	233.88	235.29	235.23	235.50	233.64	234.19	235.47	241.56
Chi-square	0.36	0.88	0.33	0.27	0.53	0.66	1.17	0.50	8.01
P-value	0.95	0.93	0.96	0.97	0.91	0.96	0.88	0.92	0.09
BMD	2.76	2.03	2.78	2.86	2.66	2.34	1.87	2.62	0.96
BMDL	1.65	1.71	1.77	1.89	1.34	1.34	1.59	1.53	0.74

AIC, Akaike's information criterion

The models were fitted using the BMDS program and a BMR of 10%; the values are in the units of milligrams per kilogram of body weight per day. The multistage model gave the lowest BMDL of the models with acceptable fits and is used for graphical presentation, as shown in Figure 1. The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

Figure 1
BMD and BMDL from the multistage model



Note: The lower line is the fit of the model to the experimental data. The vertical bars are the confidence intervals around the experimental data. The upper line is the upper bound for the response from which the lower confidence bound of the BMD (BMDL) can be defined.

2.3 Dietary exposure estimates in epidemiological studies

The Committee noted that epidemiological studies sometimes rely on responses to a food frequency questionnaire (FFQ) to estimate dietary exposure to a chemical contaminant (see section 3.1). An important limitation in the use of FFQ responses for this purpose is the potential for random exposure misclassification (also referred to as non-differential exposure misclassification). This is a non-systematic error, in that dietary exposure to the contaminant will be overestimated for some individuals and underestimated for others, but the direction and magnitude of the error are unrelated to true dietary exposure to the contaminant. Several factors contribute to this error:

- An FFQ designed to assess consumption patterns or to estimate nutrient intake might not be well suited to estimate dietary exposure to a contaminant because of the ways in which foods are grouped into categories or if the FFQ was not designed to capture information about aspects of food preparation that can affect contaminant concentration.
- An FFQ provides data only on the frequency with which a respondent consumes a particular food during a specified interval. If no information on portion size is requested from the respondent, the frequency of consumption needs to be converted to an amount of food consumed by use of standard portion sizes.
- The concentration of a contaminant in samples of a particular food is defined by a distribution rather than by a single value. The larger the variance of this distribution, the greater the error in estimating dietary exposure to a contaminant if a single (e.g. average) concentration is assigned to each food consumed.

Under most circumstances, random exposure misclassification will decrease the statistical power of hypothesis testing and bias effect estimates, such as a relative risk or an odds ratio, towards the null value (i.e. indicating the absence of association). In other words, even if a true association exists between exposure to the contaminant and the risk of an adverse health outcome, the magnitude of the association derived using FFQ responses will tend to underestimate the true magnitude of the association and to estimate it with less precision (i.e. produce a wider confidence interval). This will increase the risk of a Type II error of inference (i.e. a false negative).

As long as mean dietary exposures are estimated correctly (i.e. the errors are not skewed in either direction), exposure misclassification will not greatly influence the dose–response relationship. However, because values in the lowest exposure category (and sometimes also in the highest exposure category) are bounded only in one direction, the most common impact of exposure misclassification is that the dose–response relationship will appear to be

flatter than it really is, particularly at the low end of exposure. Background response rates and outcomes for low-dose groups will tend to be overestimated, whereas rates at high doses may be underestimated. If the degree to which exposure misclassification occurs is known, it is possible to represent the potential impact of misclassification on dose–response modelling by conducting a bootstrap analysis in which each individual dose is treated as a source of uncertainty.

When evaluating the results of studies in which FFQ responses provided the basis for estimates of dietary exposure to a contaminant, the extent to which random exposure misclassification might have influenced the conclusions drawn must be considered.

3. The toxicological, epidemiological and dietary exposure evaluation of compounds on the agenda

The Committee considered two food contaminants for the first time and re-evaluated four others. Information on the safety evaluations is summarized in Annex 2.

3.1 Acrylamide

Explanation

Acrylamide (CH_2=CHCONH_2, Chemical Abstracts Service [CAS] No. 79-06-01) is a water-soluble vinyl monomer that is formed in many common foods during cooking. Acrylamide is also a component of tobacco smoke. It is readily polymerizable. Polyacrylamide has multiple applications in chemical and manufacturing industries—for example, as a flocculant for clarifying drinking-water, as a sealant for construction of dams and tunnels, as a binder in the paper and pulp industry and in dye synthesis.

The sixty-fourth meeting of the Committee (Annex 1, reference *176*) evaluated dietary acrylamide and recommended that:

- acrylamide should be re-evaluated once the results of the planned study of carcinogenicity and long-term studies of neurotoxicity become available;
- work should continue on physiologically based pharmacokinetic (PBPK) modelling to better link biomarkers in humans with dietary exposure assessments and toxicological effects in experimental animals;
- work to reduce exposure to acrylamide in food by minimizing its concentrations should continue;
- information on the occurrence of acrylamide in food consumed in developing countries would be useful to conduct a dietary exposure assessment and consider appropriate mitigation strategies to minimize acrylamide concentrations in food.

At its present meeting, the Committee reconsidered the studies described in the monograph of the sixty-fourth meeting (Annex 1, reference *177*). New

information on occurrence and mitigation as well as dietary exposure was considered. Additionally, the Committee considered the recently completed toxicity studies, which included studies on metabolism, genotoxicity and neurodevelopmental effects following exposure to acrylamide as well as long-term toxicity and carcinogenicity studies on acrylamide and glycidamide. There were also many new epidemiological studies available for review.

Absorption, distribution, metabolism and excretion

Since the metabolism of acrylamide was last reviewed by the Committee at its sixty-fourth meeting, a number of studies have compared acrylamide metabolism in rodents and humans. Rodents and humans metabolize acrylamide to a chemically reactive epoxide, glycidamide, in a reaction catalysed by cytochrome P450 2E1 (CYP2E1). In humans, there is considerable variability in the extent of acrylamide conversion to glycidamide. This difference appears to be related to interindividual variability in the amount of CYP2E1 rather than to an enzyme polymorphism. Although there are species differences in hepatic CYP2E1 activity, PBPK modelling suggests only modest differences in biotransformation between rats and humans. Glycidamide may be further metabolized by epoxide hydrolase to glyceramide or by conjugation to glutathione, or it may react with proteins, including haemoglobin, or with deoxyribonucleic acid (DNA). Acrylamide is extensively conjugated with glutathione to form a mercapturic acid, *N*-acetyl-*S*-(2-carbamoylethyl)-L-cysteine, in all species examined and is oxidized to its corresponding sulfoxide in humans only. PBPK modelling of acrylamide metabolism and disposition has provided estimates of internal exposure to both acrylamide and glycidamide that facilitate comparisons of internal dosimetry for use in risk assessment for neurotoxicity and carcinogenicity.

Toxicological data

Despite overt symptoms of neurotoxicity (i.e. hind limb paralysis) at the highest oral acrylamide dose tested (44 mg/kg body weight [bw] per day in drinking-water), a short-term study in adult male rats indicated that only minor changes were seen in messenger ribonucleic acid (mRNA) levels of the more than 50 genes directly related to the cholinergic, noradrenergic, gamma-aminobutyric acid–releasing (GABAergic) or glutamatergic neurotransmitter systems in the striatum, substantia nigra or parietal cortex. No evidence of axonal, dendritic or neuronal cell body damage or microglial activation was found in the forebrain at acrylamide doses below 44 mg/kg bw per day. In addition, levels of serotonin, dopamine and their metabolites were essentially unchanged in the striatum, substantia nigra or parietal cortex. The motor

deficits observed were interpreted as being caused by damage to the brain stem, spinal cord and peripheral neurons.

The effect of orally administered acrylamide on neurodevelopment in rats was investigated following exposure during gestation and postnatally in two separate studies. In one study, food-motivated behaviour, evaluated at 6–12 weeks of exposure, was significantly changed only at the highest dose tested (5 mg/kg bw per day).

In a second study in rats, oral acrylamide doses of 7.9 mg/kg bw per day and 14.6 mg/kg bw per day caused gait abnormalities in dams from postnatal day (PND) 18 and PND 2, respectively, to PND 21. A corresponding reduction in pup body weight occurred over the same time interval. Histopathological changes were observed in ganglion cells of the trigeminal nerves at doses of 7.9 mg/kg bw per day and above. Pups from untreated dams that received acrylamide intraperitoneally at a dose of 50 mg/kg bw 3 times a week from PND 2 to PND 21 showed similar trigeminal nerve lesions. Morphometric data of the sciatic nerve in dams but not their pups at 14.6 mg/kg bw per day showed a significant increase in the number of degenerated small-diameter axons and myelinated nerves. Similar lesions were found in pups treated intraperitoneally. All male pups from dams treated at 14.6 mg/kg bw per day and those treated intraperitoneally showed evidence of delayed spermatogenesis.

Significantly increased incidences of neurotoxicity, measured as peripheral nerve (sciatic) axon degeneration by microscopic histopathology, were observed in a 2-year bioassay (National Center for Toxicological Research [NCTR]/National Toxicology Program [NTP] of the USA) (*3*) with F344 rats treated with acrylamide in drinking-water. The no-observed-adverse-effect levels (NOAELs) were 0.67 mg/kg bw per day in males and 1.88 mg/kg bw per day in females.

Genotoxicity

In accord with the previously reported findings, the new in vitro genotoxicity studies indicate that acrylamide in the absence of activation is a weak mutagen but an effective clastogen. In contrast, glycidamide is a mutagen and clastogen. Assays of mutagenicity in vivo have demonstrated that administration of acrylamide or glycidamide in the drinking-water increases mutant frequencies in lymphocyte *Hprt* and liver and lung *cII* genes of adult Big Blue mice by inducing primarily guanine:cytosine (G:C) to thymine:adenine (T:A) transversions. Similarly, acrylamide and glycidamide (approximately 3–5 mg/kg bw per day) are mutagenic in thyroid, but not liver or mammary gland, of male and female Big Blue rats. In addition, glycidamide, but not acrylamide, was found to be a DNA-reactive mutagen in neonatal Tk mice at

Hprt and *Tk* loci. In mice treated with acrylamide for 28 days, there was a linear increase in the number of micronuclei that achieved significance at 6 mg/kg bw per day in erythrocytes and at 4 mg/kg bw per day in reticulocytes. Use of an internal marker of acrylamide exposure, such as concentrations of haemoglobin adducts (glycidamide–valine [GA-Val], acrylamide–valine [AA-Val]) or DNA adducts (N7-glycidamide–guanine [N7-GA-Gua]), gave a better fit than the external dose for modelling micronuclei frequency. The fitted model gave a threshold at adduct levels equivalent to an external dose of 1–2 mg/kg bw per day.

Carcinogenicity

In the recently completed 2-year NCTR/NTP studies in which mice and rats were treated with acrylamide in drinking-water (*3*), the sites of tumours (thyroid and mammary gland, peritesticular mesothelium) induced in male and female F344 rats at a dose range up to 2.78 mg/kg bw per day in males and 4.09 mg/kg bw per day in females were concordant with those found in previous 2-year studies in rats. Additional tumour sites observed in the new study were heart schwannomas and pancreatic islet tumours in males. A notable absence in the new study was the lack of significantly elevated incidences of brain and spinal cord tumours of glial origin. The new study also reported the tumorigenesis of acrylamide in multiple tissues of male and female B6C3F1 mice (lung, Harderian gland, forestomach, mammary, ovary) using the same drinking-water concentrations as used in the rat study. The achieved acrylamide doses in mice were up to 9.11 mg/kg bw per day for males and 9.97 mg/kg bw per day for females. These findings were further supported by results from parallel groups of animals that were treated with equimolar concentrations of glycidamide in drinking-water. Most tumour sites at which the incidence was significantly elevated in rats and mice exposed to acrylamide were also significantly increased by glycidamide, with glycidamide-induced tumour incidences being either similar or higher. The only exceptions were ovarian benign granulosa cell tumours in female mice and pancreatic adenomas and carcinomas in male rats. Tumours in other tissues were observed to be significantly increased in glycidamide-treated rats and mice, including skin in mice and oral cavity and mononuclear cell leukaemia in rats. The concordance of tumour sites and glycidamide internal dosimetry from PBPK modelling between acrylamide- and glycidamide-treated rodents provides strong support for the hypothesis that glycidamide is the ultimate carcinogenic species derived from metabolism of acrylamide. Additional support for the tumorigenicity of glycidamide, but not acrylamide, was observed in livers of male Tk mice treated neonatally on PNDs 1, 8 and 15 and evaluated after 1 year of life.

Observations in humans

The updated analyses of workers exposed to acrylamide by inhalation revealed considerably lower relative risks for mortality from pancreatic cancer than in previous analyses of the same cohorts, and the results were not statistically significant. The updated analyses are based upon comparisons with mortality in the general population as well as comparisons of different levels of acrylamide exposure within the cohort, with control for smoking history. Taken together, in spite of high acrylamide exposure in some workers, results for these two cohorts do not provide support for any relationship between acrylamide exposure at the workplace and cancer mortality.

The potential association between dietary exposure to acrylamide and cancer has been assessed in five prospective studies. Without taking into account subgroup analyses (i.e. different histological types of tumours in a particular organ/site, different stage at diagnosis, stratified analysis by smoking), these cohorts provided 23 estimates of relative risk for 16 tumour sites. No statistically significant associations were found between dietary acrylamide exposure and the following cancers: breast (four studies), ovary (two), endometrium (two), prostate (two), urinary bladder, colon and rectum (two), stomach, oesophagus, pancreas, lung (men), brain, oral cavity, pharynx, larynx and thyroid. Statistically significant associations were found in some studies for some cancers, including renal cell cancer, when adjusted for smoking and for ovarian and endometrial cancers among non-smokers. A significant increase in risk was also reported for cancer of the oral cavity, but this was restricted to female non-smokers. For lung cancer, there was a significant inverse association among women; this association was stronger among non-smokers and for adenocarcinomas. To date, none of these associations between acrylamide exposure and cancer at particular sites have been confirmed.

No association was found between concentrations of the biomarker AA-Val haemoglobin adduct and prostate cancer in a population-based case–control study. In a prospective study, no association between AA-Val/GA-Val concentrations and risk of breast cancer in postmenopausal women was found. However, a significantly increased risk was reported in smokers after adjusting for duration and intensity of smoking. This effect was even stronger when the analysis was restricted to cases with estrogen receptor positive tumours. These associations were found for AA-Val adducts but not for GA-Val adducts.

Overall, the epidemiological studies do not provide any consistent evidence that occupational exposure or dietary exposure to acrylamide is associated with cancer in humans. Although some studies indicate an association with some tumour types, particularly the hormone-related cancers in women, this

needs confirmation. While the epidemiological investigations have not shown an increased cancer risk from acrylamide exposure, the statistical power and potential for misclassification of acrylamide dietary exposure in these studies are of concern. The reviewed studies, including those with a relatively large sample size, had low power (always below 50%) to detect an increased risk of small magnitude. Data from FFQs, which are used to estimate the extent of dietary exposure to acrylamide in population-based studies, have been shown to correlate poorly with biomarkers of acrylamide and glycidamide exposure. Dietary exposure estimates derived from FFQs cannot readily capture the inherent variability of acrylamide concentrations in individual foods (see section 2.3). Consequently, epidemiological studies that use FFQs have a limited ability to detect an association between the surrogate measure of dietary acrylamide exposure and a modest increase in cancer risk.

Analytical methods

Reliable methods for the determination of acrylamide in all relevant foods are available, as demonstrated both by collaborative validation trials of single methods as well as by proficiency tests with a variety of methods. Analytical laboratories are enabled to demonstrate and maintain measurement quality through the availability of certified reference materials and proficiency testing schemes. Isotope-labelled acrylamide for use as an internal standard is commercially available. A majority of validated and fit-for-purpose methods are isotope dilution mass spectrometric procedures, most commonly liquid chromatography–tandem mass spectrometry (LC-MS/MS) and, after derivatization, gas chromatography with mass spectrometry (GC-MS) or GC-MS/MS. Development of simpler, inexpensive and quick methods (e.g. immunoassays) has been reported, but validated methods of this type are still not available.

Formation during cooking and heat processing

The main route for acrylamide formation in foods is the Maillard reactions. Upon heating, the free amino acid asparagine is decarboxylated and deaminated to form acrylamide via routes involving initial reaction with reducing sugars or other carbonyl compounds. The Maillard reactions are also responsible for the flavour and colours typical of fried foods; unlike acrylamide formation, these processes also involve amino acids other than asparagine.

Other formation mechanisms have been identified; for example, acrylamide can be formed through pyrolysis of the wheat protein gluten or via initial enzymatic decarboxylation of asparagine in raw potatoes. Although these routes are believed to be of minor importance, the degree to which they contribute to acrylamide formation in different foods has not yet been thoroughly investigated.

Prevention and control

Reduction and control of acrylamide in foods have relied mainly on voluntary actions by the food industry to reduce the acrylamide levels in their products. Many national authorities provide information to consumers on how to reduce the formation of acrylamide in home cooking; to some extent, dietary advice is also given. A Code of Practice for the Reduction of Acrylamide in Foods has recently been adopted by the Codex Alimentarius Commission. The European Commission, in cooperation with the food industry, has initiated several measures on acrylamide mitigation. These were to a large extent based on the more extensive "toolbox for acrylamide mitigation" produced by the food industry.

Although a large and growing number of mitigation methods are being published, there is still no single method that can efficiently lower the levels of acrylamide in all foods. The food industry toolbox lists a number of measures that may be introduced at the various stages: agronomical, recipe, processing and final preparation. Only a limited number of measures have been implemented at an industrial production scale so far, including control of sugar levels in potatoes, treatment with the enzyme asparaginase, addition of various salts and acids, control of thermal input and cooking profile, and control of moisture and browning in the final product.

Significant mitigation achievements were reported by producers of potato crisps (USA = chips) and potato chips (USA = french fries) in some countries during the first years after the discovery of acrylamide in foods in 2002, but fewer achievements have been reported in recent years. Average acrylamide levels in German potato crisps produced from stored potatoes were in the range of 800–1000 µg/kg in 2002–2003 and 400–600 µg/kg in 2004–2009. In general, mitigation efforts have had limited success when applied to bread and other cereal products, although significant reductions in acrylamide levels have been reported more recently for some specific products. Mitigation after 2003 has been reported mainly for food types with comparably high acrylamide levels or single products that are at the high end of contamination within their food type. Although this might significantly reduce the exposure for some individuals or population subgroups, it will have little effect on the dietary exposure for the general population in most countries.

Levels and patterns of contamination in food commodities

At the current meeting, the Committee reviewed data from 31 countries on the occurrence of acrylamide in different foods analysed between 2004 and 2009. The total number of analytical results (single or composite samples) was 12 582, with 61% coming from Europe, 28% from Asia, 9% from North America, 1% from the Pacific and 1% from Latin America. No data were

received from Africa. The Committee noted that the occurrence data evaluated at its present meeting were more comprehensive than the data submitted at the sixty-fourth meeting. Most countries used validated analytical methods and employed quality control programmes to ensure the reliability of the data.

National mean concentrations of acrylamide in major foods were found to range from 399 to 1202 µg/kg for potato crisps (USA = chips); from 159 to 963 µg/kg for potato chips (USA = french fries); from 169 to 518 µg/kg for biscuits (USA = cookies); from 87 to 459 µg/kg for crispbread and crackers; and from 3 to 68 µg/l for coffee (ready to drink). The Committee noted that the mean concentration ranges of acrylamide in the above foods are similar to those considered in its previous evaluation at the sixty-fourth meeting. In comparing global mean acrylamide levels for commodity groups with the levels obtained at the sixty-fourth meeting, the Committee noted that acrylamide levels in rye products had decreased significantly. No significant differences were observed for products made from potato, barley, rice, wheat, maize or oats.

Food consumption and dietary exposure assessment

Data on dietary exposure for eight countries were evaluated at this meeting. All regions were represented, except for Africa, for which no dietary exposure data were available. National dietary exposures were calculated mainly using a deterministic assessment. The modelling combined national individual consumption data with mean occurrence data obtained from national monitoring surveys and with the consumer body weights reported in consumption surveys.

Estimates of mean dietary exposures at the national level ranged from 0.2 to 1.0 µg/kg bw per day for the general adult population. For adult consumers at the high (95th–97.5th) percentile, the estimates of dietary exposure ranged from 0.6 to 1.8 µg/kg bw per day. Based on the few data available for children, it was noted that children had dietary exposures to acrylamide that were about twice those of adult consumers when expressed on a body weight basis. The Committee noted that these estimates were similar to those used in the assessment performed by the sixty-fourth meeting, at which a dietary exposure to acrylamide of 1 µg/kg bw per day was taken to represent the mean for the general population and a dietary exposure of 4 µg/kg bw per day was taken to represent consumers with a high dietary exposure.

The major foods contributing to the total mean dietary exposures for most countries were potato chips (USA = french fries) (10–60%), potato crisps (USA = chips) (10–22%), bread and rolls/toast (13–34%) and pastry and sweet biscuits (USA = cookies) (10–15%). Generally, other food items contributed less than 10% to the total dietary exposures. The Committee noted

that these contributions to overall exposures were consistent with the major contributing foods identified by the sixty-fourth meeting.

International estimates of dietary exposure were prepared by combining the international means of contamination levels reviewed at this meeting with food consumption data from the Global Environment Monitoring System – Food Contamination Monitoring and Assessment Programme (GEMS/Food) consumption cluster diets (see Annex 3), which differentiate 13 regional dietary patterns for food commodities (e.g. the consumption of cassava has been combined with mean acrylamide levels taken from cassava, raw/boiled, and from processed cassava products). The Committee noted that these estimates were more refined than those prepared at the sixty-fourth meeting, which were based on the then-available five GEMS/Food regional consumption diets.

The Committee estimated the international mean dietary exposures to range between 1.1 and 4.8 µg/kg bw per day across the 13 GEMS/Food consumption cluster diets, assuming a body weight of 60 kg. Cereals and root- and tuber-based foods were the main contributors to the total dietary exposure calculations for each cluster diet. Dietary exposures from cereal-based foods are between about 0.5 and 2.8 µg/kg bw per day. Depending on the patterns of consumption in each cluster, processed foods based on wheat, maize and rice were the main commodities contributing to overall exposure from cereal-based foods. Dietary exposures from roots and tubers ranged from 0.2 to 2.2 µg/kg bw per day. Processed potato was the main contributor to overall dietary exposure in most cluster diets. Food commodities based on peas, cassava and plantain were also major contributors for some cluster diets, specifically clusters A and J. Other GEMS/Food commodities contributed less than 10% to the total dietary exposure estimations.

The Committee recognized that it was difficult to have a clear picture of national trends in dietary exposures since the last evaluation and noted that this was mainly due to the lack of updated dietary exposure data from the countries evaluated at the previous meeting. Additionally, there were differences in methodologies used in evaluations within a single country for obtaining data on consumption and occurrence. Nevertheless, when comparing international dietary exposure data with the occurrence data from the sixty-fourth and the present meetings (overall 18 000 analytical data), no significant differences were seen.

The Committee concluded that, overall, no major changes in dietary exposures had occurred since the last evaluation. Therefore, based on national and regional estimates, a dietary exposure to acrylamide of 1 µg/kg bw per day could again be taken to represent the mean for the general population, including children, and a dietary exposure of 4 µg/kg bw per day could again be taken to represent consumers with a high dietary exposure.

Dose–response analysis

At its sixty-fourth meeting, the Committee noted that the lowest NOAEL for a non-carcinogenic end-point was 0.2 mg/kg bw per day. This end-point was based on the induction of morphological nerve changes in rats following administration of acrylamide in drinking-water. There were no new studies in laboratory animals in which non-carcinogenic effects were observed at a dose below 0.2 mg/kg bw per day.

The Committee considered that the pivotal effects of acrylamide were its genotoxicity and carcinogenicity. As expressed in the previous evaluation, the Committee considered that the available epidemiological data were not suitable for a dose–response analysis. Therefore, the assessment was based on the available studies in laboratory animals. In the dose–response analysis using the USEPA BMD software (BMDS version 2.0), the nine different statistical models were used to fit the new experimental data in mice and rats from the NCTR/NTP studies (*3*). Those models resulting in acceptable fits, based on biological and statistical considerations, were selected to derive a BMD and a BMDL for a 10% extra risk of tumours (i.e. a BMD_{10} and a $BMDL_{10}$).

This process resulted in a range of BMD_{10} and $BMDL_{10}$ values for each end-point considered (Tables 2 and 3). The Committee noted that the $BMDL_{10}$ values from the NCTR/NTP 2-year bioassay of acrylamide in male and female F344 rats (*3*) were similar to those reported at the sixty-fourth meeting for the earlier rat bioassays of carcinogenicity. However, the lowest range of $BMDL_{10}$ values was observed for the Harderian gland in B6C3F1 mice treated with acrylamide. As humans have no equivalent organ, the significance of these benign mouse tumours in the Harderian gland is difficult to interpret with respect to humans. However, in view of acrylamide being a multisite carcinogen in rodents, the Committee was unable to discount the effect in the Harderian gland.

The Committee considered it appropriate to use 0.18 mg/kg bw per day (the lowest value in the range of $BMDL_{10}$ values) for tumours in the Harderian gland of male mice and 0.31 mg/kg bw per day for mammary tumours in female rats as the PODs.

Table 2
Summary of results of dose–response modelling for induction of selected tumours in rats given drinking-water containing acrylamide

Species	Sex	Neoplasm	BMD_{10} (mg/kg bw per day)	$BMDL_{10}$ (mg/kg bw per day)
F344 rats	Male	Testicular mesothelioma	2.14–2.26	1.25–1.73
		Heart malignant schwannoma	2.48–2.77	1.29–1.92
		Pancreatic islet adenoma	2.82–3.52	1.60–2.20
		Pancreatic islet adenoma or carcinoma	2.84–3.11	1.46–2.01
		Thyroid gland follicular cell carcinoma	2.03–2.62	1.11–1.83
		Thyroid gland follicular cell adenoma or carcinoma	3.65–4.67	2.31–2.54
F344 rats	Female	Clitoral gland carcinoma	4.31–5.19	1.55–3.11
		Mammary gland fibroadenoma	0.58–1.35	0.31–0.87
		Mammary gland fibroadenoma or adenocarcinoma	0.62–1.41	0.33–0.90

BMD_{10}, benchmark dose for 10% extra risk of tumours; $BMDL_{10}$, 95% lower confidence limit for the benchmark dose for 10% extra risk of tumours. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

Table 3
Summary of results of dose–response modelling for induction of selected tumours in mice given drinking-water containing acrylamide

Species	Sex	Neoplasm	BMD_{10} (mg/kg bw per day)	$BMDL_{10}$ (mg/kg bw per day)
B6C3F1 mice	Male	Harderian gland adenoma	0.36–0.67	0.18–0.56
		Harderian gland adenoma or carcinoma	0.37–0.66	0.18–0.55
		Lung alveolar/bronchiolar adenoma	2.14–4.15	1.29–2.84
		Lung alveolar/bronchiolar adenoma or carcinoma	2.13–4.07	1.28–2.78
		Forestomach squamous cell papilloma	4.82–8.09	3.18–6.02
		Forestomach squamous cell papilloma or carcinoma	3.96–6.82	2.68–5.36
B6C3F1 mice	Female	Harderian gland adenoma	0.43–0.63	0.31–0.53
		Lung alveolar/bronchiolar adenoma	1.95–4.00	1.29–2.84
		Lung alveolar/bronchiolar adenoma or carcinoma	2.02–3.84	1.28–2.78
		Mammary gland adenocarcinoma	1.61–4.08	1.19–3.41
		Mammary gland adenoacanthoma	10.92–11.12	6.39–8.19
		Mammary gland adenocarcinoma or adenoacanthoma	2.91–9.04	2.06–5.22
		Ovarian benign granulosa cell tumour	9.45–11.45	6.51–7.83

BMD_{10}, benchmark dose for 10% extra risk of tumours; $BMDL_{10}$, 95% lower confidence limit for the benchmark dose for 10% extra risk of tumours. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

Evaluation

The Committee noted that mitigation after 2003 has been reported for food types with high acrylamide levels or single products that contain higher levels within their food type. Although this might significantly reduce the exposure for some individuals or population subgroups, the Committee noted that this will have little effect on the dietary exposure of the general population in all countries. In line with this, neither the estimated average acrylamide exposure for the general population (0.001 mg/kg bw per day) nor the exposure for consumers in the high percentile (0.004 mg/kg bw per day) had changed since the sixty-fourth meeting. The MOE calculated relative to the NOAEL of 0.2 mg/kg bw per day for the most sensitive non-carcinogenic end-point—namely, morphological changes in nerves, detected by electron microscopy, in rats—therefore remains unchanged. For the general population and consumers with high exposure, the MOE values are 200 and 50, respectively. Consistent with the conclusion made at the sixty-fourth meeting, the Committee noted that while adverse neurological effects are unlikely at the estimated average exposure, morphological changes in nerves cannot be excluded for individuals with a high dietary exposure to acrylamide.

When average and high dietary exposures are compared with the $BMDL_{10}$ of 0.31 mg/kg bw per day for the induction of mammary tumours in rats, the MOE values are 310 and 78, respectively. For Harderian gland tumours in mice, the $BMDL_{10}$ is 0.18 mg/kg bw per day, and the MOE values are 180 and 45 for average and high exposures, respectively.

The Committee considered that for a compound that is both genotoxic and carcinogenic, these MOEs indicate a human health concern. The Committee recognized that these MOE values were similar to those determined at the sixty-fourth meeting and that the extensive new data from cancer bioassays in rats and mice, PBPK modelling of internal dosimetry, a large number of epidemiological studies and updated dietary exposure assessments support the previous evaluation.

The Committee noted that there was a poor correlation between the estimated dietary exposure and internal biological markers of acrylamide exposure (AA-Val and GA-Val adducts) in humans and that worker cohort epidemiological studies did not provide any evidence that exposure to acrylamide resulted in an increase in the incidence of cancer. To better estimate the risk from acrylamide in food for humans, the Committee recommended that longitudinal studies on intra-individual levels of acrylamide and glycidamide haemoglobin adducts be measured over time in relation to concurrent dietary exposure. Such data would provide a better estimate of acrylamide exposure for epidemiological studies designed to assess risk from the diet.

A detailed addendum to the monograph was prepared.

Recommendation

The Committee recommends further efforts on developing and implementing mitigation methods for acrylamide in foods of major importance for dietary exposure.

3.2 Arsenic

Explanation

Arsenic is a metalloid that occurs in different inorganic and organic forms, which are found in the environment both from natural occurrence and from anthropogenic activity. Arsenic was previously evaluated by the Committee at its tenth, twenty-seventh and thirty-third meetings (Annex 1, references *13*, *63* and *84*). At its twenty-seventh meeting (1983), it was concluded that "on the basis of the data available the Committee could arrive at only an estimate of 0.002 mg/kg b.w. as a provisional maximum tolerable daily intake for ingested inorganic arsenic; no figure could be arrived at for organic arsenicals in food" (Annex 1, reference *63*). This was based on the observation that arsenicism can be associated with water supplies containing an upper arsenic concentration of 1 mg/l or greater and that a concentration of 0.1 mg/l may give rise to presumptive signs of toxicity. Assuming a daily water consumption of 1.5 litres, the Committee concluded that inorganic arsenic intakes of 1.5 mg/day were likely to result in chronic arsenic toxicity and that daily intakes of 0.15 mg may also be toxic in the long term to some individuals. The Committee noted that the International Programme on Chemical Safety (IPCS) had estimated that an arsenic concentration of 0.2 mg/l in drinking-water would lead to a 5% lifetime risk of skin cancer, but that skin cancer did not occur in the absence of other toxic effects due to arsenic. The Committee also noted a need for information on:

- arsenic accumulation in humans exposed to various forms of arsenic in the diet and drinking-water;
- the identification, absorption, elimination and toxicity of arsenic compounds in food, with particular reference to arsenic in fish;
- the contribution of arsenic in fish to human body burden of arsenic;
- epidemiological studies on populations exposed to elevated intakes of arsenic of known speciation.

At its thirty-third meeting (1988), the Committee considered information relevant to assessing the significance of organoarsenicals in fish. The previous evaluation was confirmed by assigning a provisional tolerable weekly intake (PTWI) of 0.015 mg/kg bw for inorganic arsenic, "with the clear understanding that the margin between the PTWI and intakes reported to have toxic effects in epidemiological studies was narrow" (Annex 1, reference *84*). The

Committee noted that the organic forms of arsenic present in seafood needed different consideration from the inorganic arsenic in water. It concluded that there had been no reports of ill-effects among populations consuming large quantities of fish that result in organoarsenic intakes of about 0.05 mg/kg bw per day, but further investigation would be desirable to assess the implications for human health of exposure to naturally occurring organoarsenic compounds in marine products.

Inorganic arsenic has also been evaluated on a number of occasions by the International Agency for Research on Cancer (IARC). In 1973, IARC concluded that there was a causal relationship between skin cancer and exposure to inorganic arsenic in drugs, in drinking-water with a high arsenic content or in the occupational environment and that the risk of lung cancer was clearly increased in certain smelter workers who inhaled high levels of arsenic trioxide. However, the causative role of arsenic was uncertain, as the influence of other constituents of the working atmosphere could not be determined. In 1980, IARC concluded that there was sufficient evidence that inorganic arsenic compounds are skin and lung carcinogens in humans (Group 1). In 2004, IARC concluded that there was sufficient evidence in humans that arsenic in drinking-water causes cancers of the urinary bladder, lung and skin, whereas the evidence for carcinogenicity in experimental animals was limited. In 2009, IARC again concluded that arsenic in drinking-water causes cancers of the urinary bladder, lung and skin and that the evidence was "limited" for cancers of the kidney, liver and prostate.

At its present meeting, the Committee was asked to consider all information related to the toxicology and epidemiology, exposure assessment, including biomarker studies, analytical methodology, speciation and occurrence in food and drinking-water, in order to re-evaluate and review the PTWI for inorganic arsenic. The literature relating to arsenic is extensive, and the present Committee used three recent reviews—by the United States Agency for Toxic Substances and Disease Registry, the European Food Safety Authority (EFSA) and IARC—as the starting point for its evaluation and also took into account newer studies that were considered to be informative for the evaluation. The arsenic-containing compounds found in water, foods and biological samples are shown in Table 4.

Absorption, distribution, metabolism and excretion

Absorption of arsenic depends on the chemical species and its solubility as well as the matrix in which it is present. Soluble arsenicals in water are highly bioavailable. Inorganic arsenic is rapidly cleared from blood both in humans and in most experimental animal species that have been tested; an exception is rats, in which arsenic binds to erythrocytes, delaying clearance. Inorganic arsenic is metabolized primarily by stepwise reduction of pentavalent arsenic

Table 4
Arsenic compounds found in water, foods and biological samples

Name	Synonyms and abbreviations	CAS No.
Arsenate	As^{V}	—
Arsenite	As^{III}	—
Methylarsonic acid	Monomethylarsonic acid, methylarsonate, MMA^{V}	124-58-3
Dimethylarsinic acid	Dimethylarsinite, cacodylic acid, DMA^{V}	75-60-5
Methylarsonous acid	Monomethylarsonous acid, MMA^{III}	—
Dimethylarsinous acid	DMA^{III}	—
Arsenobetaine	AB	64436-13-1
Arsenocholine	AC	39895-81-3
Trimethyl arsine oxide	TMAO	4964-14-1
Tetramethylarsonium ion	TMA^{+}	27742-38-7
Dimethylarsionylethanol	DMAE	—
Trimethylarsoniopropionate	TMAP	—
Dimethylarsionylribosides	Oxo-arsenosugars	—
Dimethylmonothioarsinic acid	$DMMTA^{V}$	—
Dimethyldithioarsinic acid	$DMDTA^{V}$	—

Note: Except for biochemical and toxicological studies of specific arsenic compounds, the valency of MMA and DMA is usually not specified. The analysis of MMA^{III} and DMA^{III} has become possible only recently. In this report, the terms MMA and DMA are used as cited in the original papers. Where MMA and DMA are measured in foods, they have been measured as the pentavalent form. Where biological samples have been analysed, it is assumed that MMA and DMA refer to total [MMA^{III} + MMM^{V}] and total [DMA^{III} + DMM^{V}], respectively.

(arsenate) to trivalent arsenic (arsenite) followed by oxidative addition of methyl groups, although alternative pathways have also been proposed that include methylated arsenical glutathione metabolites. Most ingested arsenic species are excreted via the kidney within a few days. Ingested inorganic arsenic is excreted as inorganic arsenate and arsenite and as the pentavalent methylated metabolites MMA^{V} and DMA^{V}, with lesser amounts of the trivalent methylated metabolites MMA^{III}, DMA^{III} and thioarsenical metabolites. Whereas it has previously been assumed that methylation of inorganic arsenic was a detoxification route, it is not entirely clear whether or not this is correct, because, based on limited in vitro and in vivo data, MMA^{III} and DMA^{III} appear to be more toxic than inorganic arsenic and have high affinity for thiols and cellular proteins.

Major organic arsenicals present in fish when ingested undergo very little biotransformation and are excreted almost entirely unchanged. However, some organoarsenicals, such as arsenolipids present in cod liver and arsenosugars in mussels and algae, can be metabolized to DMA^{V} when ingested.

Toxicological data

Arsenic toxicity depends on the chemical form and its solubility and varies among animal species and with route of administration. Generally, trivalent arsenic is more toxic than the pentavalent forms. Oral administration of inorganic arsenicals to laboratory animals has a number of effects, including effects on the cardiovascular, respiratory, gastrointestinal, haematological, immune, reproductive and nervous systems. MMA^V administration to experimental animals has been shown to have effects on the gastrointestinal tract, kidney, thyroid and reproductive system, with the effect seen at the lowest doses being diarrhoea. DMA^V has effects on the urinary bladder, kidneys, thyroid and fetal development.

Studies in experimental animals conducted according to standard protocols have generally not shown increased tumour incidences following chronic oral exposure to inorganic arsenic. However, evidence of tumour promotion and co-carcinogenicity has been reported. In addition, studies involving administration of arsenite to pregnant mice in their drinking-water have shown evidence of transplacental carcinogenesis.

MMA^V has not shown evidence of carcinogenicity in 2-year cancer bioassays with doses equivalent to up to 100 mg/kg bw per day. DMA^V (administered in drinking-water at $\geq$50 mg/l) was carcinogenic in the urinary bladder of rats, but not mice. DMA^V is not genotoxic, and its carcinogenic mode of action is considered to involve cytotoxicity to the bladder epithelium and sustained increased cell proliferation; the rat is considered to be particularly sensitive to DMA^V because of slower elimination and possibly a greater potential for metabolism to DMA^{III} compared with other species. The NOAEL was equivalent to 0.73 mg/kg bw per day.

In its most recent evaluation, IARC concluded that there is sufficient evidence for carcinogenicity of inorganic arsenic compounds in experimental animals and sufficient evidence for carcinogenicity of DMA^V in experimental animals. Evidence from a wide range of studies has led to the conclusion that arsenic compounds do not react directly with DNA. There are a number of proposed mechanisms of carcinogenicity of inorganic arsenic, including oxidative damage, epigenetic effects and interference with DNA damage repair.

Because of a general lack of data on both exposure to and toxicity of organic arsenicals, the Committee further considered only inorganic arsenic for this report.

Taking into account the lack of a good animal model for carcinogenicity of inorganic arsenic compounds and the large number of data available from epidemiological studies, the Committee did not consider the data from experimental animals appropriate for the dose–response analysis.

Observations in humans

The main adverse effects reported to be associated with long-term ingestion of inorganic arsenic by humans are cancer, skin lesions, developmental effects, cardiovascular disease, neurotoxicity and diabetes.

The classification of arsenic as a carcinogen was originally based on evidence of skin cancers. Studies in Taiwan, China, and other regions where high exposures to arsenic in drinking-water occurred have confirmed the relationship. Significant associations between exposure to high levels of ingested arsenic in drinking-water and bladder cancer have been observed in ecological studies from Chile, Argentina and Taiwan, China, and cohort studies in Taiwan, China. Some of the studies showed an association only in smokers. In studies from Chile, Argentina and Taiwan, China, exposure to arsenic at high concentrations in drinking-water has been shown to be associated with lung cancer. Again, when smokers and non-smokers were compared, the associations were stronger in the smokers. Nutritional status of exposed populations has been observed to influence cancer risk. Thus, compromised nutrition (e.g. low protein intake) is likely to be associated with significantly higher risk. The evidence for an association with cancers at other sites, including prostate, liver and kidney, is less conclusive.

Epidemiological studies in different regions of the world have consistently demonstrated a strong association between long-term inorganic arsenic ingestion and skin lesions, typically in the form of hyperkeratosis, hyperpigmentation or hypopigmentation. Observations of skin lesions following low chronic exposure have suggested that these characteristic dermal changes are sensitive indications of the toxic effects of inorganic arsenic.

Available epidemiological studies indicate a positive relationship between high concentrations of inorganic arsenic in drinking-water and sensitive endpoints for peripheral and central neurotoxicity. There is some evidence that exposure of children to inorganic arsenic in areas with elevated arsenic concentrations (>50 μg/l) in drinking-water produces effects on cognitive performance, but so far this is not conclusive.

The cardiovascular outcomes that have been associated with chronic exposure to arsenic through drinking-water include blackfoot disease (BFD), increased mortality or prevalence of coronary heart disease, peripheral arterial disease, myocardial infarction and stroke, and other cardiovascular endpoints, such as increased blood pressure and prolonged QT interval of the electrocardiogram. The association between BFD and inorganic arsenic exposure has been confirmed by many studies, but BFD has been reported primarily in an area along the south-western coast of Taiwan, China, where arsenic contamination in well water is very high (170–880 μg/l). Except for

BFD, the reported associations between inorganic arsenic exposure and cardiovascular disease prevalence/mortality and other cardiovascular end-points currently do not provide sufficient evidence of causality and are not considered pivotal for the assessment.

Studies conducted in Bangladesh and Taiwan, China, indicated an extra risk of diabetes among high-exposure populations. In addition, recent findings suggest that in utero arsenic exposure impaired child thymic development and that enhanced morbidity and immunosuppression might occur. However, as a result of limitations in the studies, the relationship between arsenic exposure and these outcomes remains uncertain.

The Committee concluded that the greatest strength of evidence for a causal association between inorganic arsenic and adverse effects in humans is for cancers of the skin, urinary bladder and lung and skin lesions (hyperkeratosis, hyperpigmentation and hypopigmentation) observed in studies in which levels of arsenic in drinking-water were relatively high (e.g. ≥100 μg/l). For this evaluation, studies were preferred that included documentation of exposure from drinking-water both at higher concentrations (e.g. ≥300 μg/l) and also at relatively lower concentrations (e.g. <100 μg/l). This was in order to assess effects across a broad gradient of exposure and to avoid extrapolation below the observed range in the dose–response modelling. For skin cancer, three of the four most recent studies of low-level exposure utilized toenail arsenic as a biomarker of exposure; however, the relationship between toenail arsenic and total dietary exposure to inorganic arsenic remains uncertain. Further, as arsenic-related skin lesions may be a possible precursor to skin cancer and have been reported at lower concentrations of arsenic in drinking-water compared with skin cancer, the Committee considered the data for skin lesions to be a more sensitive adverse effect than skin cancer. Thus, pivotal data were identified from epidemiological studies reporting a positive association with arsenic exposure and these effects (i.e. cancers of the lung and urinary tract and skin lesions).

Analytical methods

The most common detection techniques for arsenic are inductively coupled plasma mass spectrometry (ICP-MS), ICP–atomic emission spectroscopy (ICP-AES) and hydride generation coupled with atomic absorption spectroscopy (HG-AAS) or atomic fluorescence spectroscopy (HG-AFS). ICP-AES is generally adequate for determination of total arsenic in foods, and its sensitivity can be improved by coupling to HG. ICP-MS has the highest sensitivity without derivatization. HG-AAS and HG-AFS have limits of detection (LODs) in the microgram per kilogram range, which is adequate for all foods. For speciation with HG-based detection systems, some organoarsenic species require oxidation to species that form volatile arsines prior to their detection.

Samples prepared for total arsenic determination are mineralized by either wet or dry methods. Microwave is the most common closed system used in wet mineralization, although temperatures higher than those that can be achieved by microwave are needed for the complete degradation of some organoarsenic species. This leads to an underestimation of total arsenic in some foods when HG-based detection systems are used. Recent developments, such as microwave-induced combustion methods, are solving this problem. In dry mineralization, addition of ashing aids is necessary to avoid arsenic losses by volatilization.

Methodological research in the last decade has been targeted to arsenic speciation. Quantitative extraction of arsenic species from food matrices is one of the main methodological problems, and efficiencies vary widely, depending on the nature of the matrix and the method used. Polar solvents assisted by ultrasound, accelerated solvent extraction or microwave are commonly used. Extraction of arsenite is especially difficult to achieve, because of binding to thiol groups in proteins. Separation of arsenic species is most commonly achieved by high-performance liquid chromatography (HPLC). Multidimensional chromatography (different columns and conditions) may be needed for samples with a large number of arsenic species; up to 23 species have been found in seaweed and seafood, for example. Further difficulties are that the elution may not be quantitative under certain conditions, and the eluent may change the arsenic oxidation state.

Most of the current work on arsenic speciation has been targeted to characterization of arsenic species profiles in food products, without special attention to inorganic arsenic. There is a current need for validated and horizontal methods for selective extraction and determination of inorganic arsenic and for certified reference materials for inorganic arsenic in foods. Further, it would be more appropriate to report total inorganic arsenic rather than arsenite and arsenate, because various extraction/analytical procedures may change the oxidation state.

Effects of processing

Peeling of vegetables and polishing of rice reduce the content of total arsenic. Washing or soaking rice and seaweed and discarding the water before cooking reduce arsenic levels, especially inorganic forms. Decreases in arsenic levels with boiling have been described for rice, pasta, seaweed and seafood products, except where the water used is contaminated with arsenic, when levels may increase. The main arsenic species solubilized are AB, DMA and arsenosugars for seafood products and inorganic arsenic for cereals and seaweed. Limited studies in which seafood was heated at temperatures above 150 °C have reported that up to 11% of AB is transformed to TMAO and TMA^+.

Prevention and control

Commercial-scale water treatment processes to remove arsenic in water are available. Simple arsenic removal systems for household wells have also been developed. Low-cost systems in arsenic-endemic areas generally utilize elemental iron, iron or aluminium oxides and carbon as adsorbents for arsenic. Many household treatment systems fail prematurely because of high levels of phosphate in water, and maintenance and disposal of arsenic-contaminated wastes are difficult. Studies in Bangladesh have shown that most rural households prefer sharing uncontaminated wells or filtering low-arsenic surface water through sand to treating groundwater. Sand filtration gives mixed results with respect to removal of biological pathogens. Spatial variability in groundwater arsenic contamination in Argentina, Chile and the river deltas of South and South-east Asia is very high, so villages usually have a mixture of contaminated and uncontaminated wells. Deeper groundwater aquifers often have low arsenic levels that can be used to develop community water supplies.

Apart from processing possibilities, practical prevention and control approaches for arsenic in foods are limited. Attempts to reduce arsenic uptake into food crops by additions of phosphate fertilizer and iron oxides have given equivocal and unconvincing results with several vegetable and cereal crops. Silicate additions to soil have been shown to reduce arsenic levels in rice grain where soils are low in silicate. Growing rice under less reducing soil conditions can dramatically reduce grain arsenic levels. However, the challenge is to do this without substantial loss of yields in uncontaminated soils. Very limited identification of "low" and "high" arsenic rice varieties has been reported, and more data are needed before recommendations can be made to farmers and consumers.

Levels and patterns of contamination in food commodities

Data on total arsenic contents of foods for evaluation at the present meeting were obtained from the literature and from data submitted to the Committee by Australia, Brazil, France, Japan, New Zealand and Singapore. The total number of analytical results (single or composite) evaluated at the present meeting was 17 498. Table 5 summarizes the ranges of total arsenic concentrations by food category, based on results with quantified values (minimum to maximum). The highest total arsenic concentrations have been found in seaweed, fish and shellfish, mushrooms and fungi, rice and rice products and some meat products. The levels in the remaining food products usually do not exceed 1 mg/kg. In some food groups, the number of non-detectable/non-quantifiable results was important (n = 9081) and influences the derivation of mean concentrations; this was the case with milk products (66%), meat and meat products (74%), eggs and egg products (65%), bakery wares (70%), cereals other than rice (80%) and vegetables other than mushrooms (86%).

Table 5
Summary of available data on total arsenic concentrations in food products

Food categories	*n*	*n* < LOR[a]	Range (mg/kg)
Diary products and analogues			
Milk and milk powder	284	65	0.001–0.15
Milk products	92	61	0.010–0.35
Fats and oils	39	0	0.003–0.18
Meat and meat products			
Meat	4977	4124	0.004–0.78
Offal	2074	1096	0.009–0.45
Meat products	50	20	0.003–3.25
Eggs and egg products	171	111	0.003–0.04
Confectionery products	186	61	0.002–1.13
Sweeteners	138	21	0.003–0.26
Bakery wares	71	49	0.002–0.25
Beverages			
Alcoholic beverages (except rice distilled spirits)	462	64	0.001–0.05[b]
Rice distilled spirits	8	2	0.050–1.64[b]
Non-alcoholic beverages	120	16	0.001–0.26[b]
Vegetables/fruits/nuts/seaweed			
Fruits	966	800	0.005–2.20
Vegetables (except mushrooms and fungi)	2503	2164	0.001–1.27
Mushrooms and fungi	302	60	0.011–5.79
Nuts and oilseeds	70	15	0.005–0.88
Dried seaweeds	953	3	0.114–236
Cereals and cereal products			
Cereals (except rice)	410	325	0.007–0.43
Rice	1693	0	0.002–1.83
Breakfast cereals	17	10	0.017–0.27
Pasta	19	9	0.003–0.18
Fish and fish products			
Marine fish	1409	0	0.10–62
Shellfish	171	0	0.090–66
Freshwater fish	238	0	0.060–4.72
Baby food products	75	5	0.001–4.66

LOR, limit of reporting (detection or quantification limit)
[a] Results presented for detected values only (not detected [ND] = 0).
[b] Data expressed as mg/l.

Table 6 summarizes the ranges of levels of inorganic arsenic obtained from the literature and from data submitted by Japan, France and Singapore (minimum to maximum). The total number of analytical (single or composites) results evaluated at the present meeting was 1737.

Levels of inorganic arsenic in foods and beverages usually do not exceed 0.1 mg/kg, with mean values generally less than 0.03 mg/kg. However, seaweed, rice and some fish and seafood commodities have higher inorganic arsenic levels, as do food crops grown in arsenic-contaminated soils.

Table 6
Summary of available data on inorganic arsenic concentrations in food products[a]

Food products	*n*	*n* < LOD	Concentration range (mg/kg)
Dried seaweed	539	4	0.1–130
Rice	837	0	0.01–0.51
Fish and fish products	325	1	0.001–1.2
Vegetables	36	1	0.008–0.61

[a] Results presented for detected values only (ND = 0).

In the seaweed *Hizikia fusiforme*, inorganic arsenic is more than 50% of total arsenic, with levels usually ranging from 30 to 130 mg/kg. In other seaweed species, inorganic arsenic is less than 15% of total arsenic, with levels normally below 2 mg/kg. The proportion of inorganic arsenic in rice varies from 17% to 100% of total arsenic and in vegetables from 33% to 74%, with maximum concentrations of 0.5 and 0.6 mg/kg, respectively. The proportion of inorganic arsenic usually does not exceed 10% of the total arsenic in fish and fish products, but it was found to reach 15% in shellfish from areas with some degree of arsenic contamination.

There are a variety of organoarsenic species in foods. For MMA and DMA, no information was available on their oxidation state in food products. In meat, DMA is the major species found in most studies, together with AB and minor amounts of MMA. In poultry meat, the presence of nitarsone, a phenylarsonic acid used as a coccidiostat, has also been reported. The greatest variety of arsenic species in vegetables has been detected in seaweeds, where arsenosugars are the major species, with smaller amounts of DMA, arsenolipids and thioarsenic compounds. Mushrooms also contain many arsenic species, including AB, MMA, TMAO, DMA, AC and TMA^+. For other vegetables, MMA has been found in carrot, radish and potato, and MMA and DMA in chard and aubergine. Arsenic species found in fish and fish products include AB, arsenosugars, MMA, DMA, AC, TMA^+, TMAO, DMAE, TMAP, arsenolipids and thioarsenic compounds. AB is the major species (80–90%), except in some kinds of shellfish, where arsenosugars are the major species found.

Food consumption and dietary exposure assessment

Dietary exposure estimates for arsenic were reported by the Committee at the twenty-seventh meeting and were not revised at the thirty-third meeting. Only values for total arsenic were given for several European countries, the USA, Canada and the Republic of Korea; these ranged from 10 to 200 µg/day from food (0.17–3.33 µg/kg bw per day, assuming a body weight of 60 kg). Estimated dietary exposures to total arsenic from water ranged from 15 to 750 µg/day (0.25–12.5 µg/kg bw per day), reflecting arsenic

concentrations in water of 10 µg/l and 500 µg/l and assuming a consumption of 1.5 litres of water per day. The Committee at the twenty-seventh meeting noted that water and seafood were the major sources of total arsenic, with other foods making minor contributions.

The focus of the Committee at the present meeting was on dietary exposure to inorganic arsenic; however, the majority of dietary exposure estimates submitted for evaluation were for total arsenic. The main factors influencing dietary exposure to inorganic arsenic are the water supply, type of food consumed and food preparation methods.

Where water is contaminated with arsenic, it is one of the most significant sources of inorganic arsenic exposure. It is also a major source of inorganic arsenic in food produced by irrigation with arsenic-contaminated water and from food preparation and cooking. Rice takes up high amounts of arsenic, but speciation of arsenic in rice varies between different regions, with a higher inorganic content in rice grown in Asia compared with the USA. Rice tends to be a major source of inorganic arsenic from food, particularly in Asia and other countries where it is a staple food. The level of inorganic arsenic in the rice consumed also varies, depending on food processing and preparation methods.

Arsenic contamination of groundwater is widespread, and there are a number of regions where arsenic contamination of drinking-water is important. Areas affected include southern Asia (e.g. Bangladesh, India), South-east and East Asia (e.g. China, including Taiwan, Mongolia, Viet Nam), the Americas (e.g. Argentina, Canada, Chile, Mexico, USA) and Europe (e.g. Finland, Hungary, Romania). Exposure to inorganic arsenic from water can be very variable, with high and low arsenic sources present in close proximity. Contaminated water that is used for drinking and food preparation would normally contain arsenic at concentrations between 10 and 200 µg/l. However, concentrations above 200 µg/l have been reported in some areas. The amount of water consumed also varies according to the region, temperature, physical activity and types of food, with soups and rice being examples of foods that will either contain high quantities of water or take up large quantities of water. This can result in a total water consumption of between 1.5 and 5 litres per day.

The fact that water consumption and water used in cooking are not always included in dietary exposure estimates also makes direct comparison of reported total and inorganic arsenic dietary exposures found in different studies difficult, as exposure will be underestimated where water has not been included. In estimating dietary exposure to inorganic arsenic, variations in the different species of arsenic within a food category and between food categories need to be considered.

A summary of reported national inorganic arsenic estimates is given in Table 7, with ranges taken from various studies for some countries. It is particularly difficult to predict dietary exposures to arsenic at a regional level due to the complex factors discussed above that influence exposure at a local level. International estimates using the 13 GEMS/Food consumption cluster diets were not generated, as the Committee considered that this level of generalization was not appropriate for estimating dietary exposures to inorganic arsenic.

In most circumstances, it would be expected that estimates of dietary exposure to inorganic arsenic using individual dietary records would be more accurate than those obtained using population food consumption figures, such as normally used in TDSs or model diets. However, it is not possible to assume this is the case; for example, the EFSA estimates for European countries used individual records but assigned inorganic arsenic values derived from conversion factors applied to total arsenic levels for broad food groups, introducing uncertainties in the estimates and tending to overestimate dietary exposure compared with individual country studies in the region.

In general, the ranges of dietary exposure to inorganic arsenic for North America and Europe were similar but were lower than those reported for countries in Asia. An exception was Bangladesh, for which mean dietary exposure to inorganic arsenic was estimated to be up to 3 times that in other Asian countries. Mean dietary exposure to inorganic arsenic for adults in a community in Chile was 7 times higher at the upper end of the reported range than that reported for adults elsewhere.

For infants and children, a limited amount of information was available for Europe and the USA; in general, estimates of dietary exposure to inorganic arsenic for children were higher than those for adults from the same population when expressed per kilogram of body weight.

With the exception of dietary exposure estimates for inorganic arsenic for Bangladesh and Chile, mean reported dietary exposures for adults or whole populations were less than 1 μg/kg bw per day, and upper-percentile dietary exposures were less than 1.5 μg/kg bw per day. For infants and children, mean dietary exposure estimates for inorganic arsenic were less than 2 μg/kg bw per day, and upper-percentile estimates were less than 3 μg/kg bw per day. The mean dietary exposures of up to 3 μg/kg bw per day for Bangladesh were for a small community known to have contaminated water; the results from the study in Chile would need to be confirmed.

For countries where rice is the staple food, rice and water were the major contributors to total inorganic arsenic dietary exposures, with wheat and vegetables being minor contributors. In Europe and North America, where

Table 7
Summary of inorganic arsenic dietary exposure estimates

Country/region	Mean exposure (µg/kg bw per day)	Upper-percentile exposure (µg/kg bw per day)
Europe		
Europe[a] (EFSA)	0.21–0.61 adult	0.36–0.99 adult (95th)
	0.31–1.39 child 1–8 years	0.61–2.66 child 1–8 years (95th)
	0.03–1.63 infant <12 months	—
Belgium[b]	0.10 all	0.16 all (90th)
France TDS[c]	0.10 adult	0.27 adult (95th)
	0.14 child 3–14 years	0.34 child 3–14 years (95th)
United Kingdom TDS[c]	0.02–0.12 adult	0.05–0.16 adult (97.5th)
	0.03–0.20 child 1–18 years	0.08–0.40 child 1–18 years (97.5th)
	0.45 infant <12 months	0.74 infant (95th)
North America		
Canada TDS[c]	0.29 all	
USA TDS, other studies[d]	0.08–0.20 adult	0.16–0.34 adult (95th)
	0.12–0.32 child 1–6 years	—
	0.24–1.19 infant <12 months	—
South America		
Chile[e]	2.08–21.48 adult	
Asia		
Bangladesh[f]	1.68–3.00 adult	
China TDS[c]	0.24–0.76 adult	
China, Province of Taiwan[g]	0.91 adult	
Japan TDS, other study[h]	0.36–0.46 adult	0.83–1.29 adult (95th)

TDS, total diet study

[a] Individual dietary records for 19 European countries, different scenarios using conversion factors, drinking-water included.

[b] Individual dietary records for Belgium, analysed inorganic values for fish and seafood commodities only, drinking-water not included.

[c] Total diet studies; France 2001–2002 TDS, 10% total arsenic assumed to be inorganic, drinking-water included; Canada 1985–1988 TDS, conversion factors applied to total arsenic, drinking-water not included; China 2007 TDS analysed inorganic arsenic, drinking-water included; United Kingdom 2006 TDS analysed inorganic arsenic, drinking-water included, previous TDSs did not.

[d] Various studies based on individual dietary records for USA from 1986–1987 Nationwide Food Consumption Survey or 1994–1996, 1998 supplement Continuing Survey on Food Intakes by Individuals (CSFII), inorganic arsenic levels from a market basket survey of inorganic arsenic in food, drinking-water included in some studies.

[e] Small community in Chile, drinking-water included, seasonal contamination of river water used as drinking-water source.

[f] Small community in Bangladesh, total arsenic reported, assumed 70% total arsenic is inorganic, drinking-water not included.

[g] Small community in Taiwan, China, rice and yams only included with analysed inorganic arsenic values, drinking-water not included.

[h] Two studies; Japan 2000 TDS, drinking-water included, conversion factors applied to total arsenic; other study of women in fishing and rice farming communities, analysed inorganic arsenic for fish, shellfish, seaweed and edible algae, Japan TDS values for other foods, drinking-water not included.

wheat-based products and potatoes are staple foods, these were major contributors to inorganic arsenic dietary exposure, as well as other vegetables, milk and meat and their products. Water can contribute up to 50% of total dietary exposure in areas in these regions where the water is not contaminated. Although total arsenic levels are higher in fish and shellfish than in other foods, consumption of fish and shellfish does not have a major influence on dietary exposure to inorganic arsenic, as the majority of arsenic in fish and in the edible portion of shellfish is organic. The exception to this is for populations (e.g. Japan) or individuals in other populations who consume high amounts of seaweed and other edible algae, some species of which are very high in inorganic arsenic and consumption of which can make a significant contribution to inorganic arsenic dietary exposure. No studies included dietary supplements, although some of these may contain appreciable amounts of inorganic arsenic, which may also mean that dietary exposures to inorganic arsenic are underestimated for individuals taking these supplements on a regular basis.

Dose–response analysis

The following studies were selected for dose–response modelling of the respective end-points. For lung cancer, data were from a recent prospective study in north-eastern Taiwan, China, of 6888 residents for whom arsenic concentrations in drinking-water had been ascertained, with an average 11.5 years of follow-up. Residents 40 years of age and older at study initiation with 178 incident lung cancer cases identified (*4*) were used for modelling. An earlier case–control study of lung cancer was not preferred for modelling due to potential selection bias in hospital-based controls. For urinary tract cancer, data from the same prospective study in north-eastern Taiwan, China, with 45 incident cases of urinary tract cancer (*5*) were used for dose–response modelling. Three arsenic-related skin lesion case–control studies were considered: two conducted in Bangladesh and one conducted in Inner Mongolia, China. Substantial differences exist among the studies in factors such as case definition, exposure assessment methods and assessment of possible confounders, including smoking and sun exposure. Considering these differences, these studies were not used for the evaluation.

The exposure metric in these studies was concentration of arsenic in drinking-water; total dietary exposure to inorganic arsenic from food and water was not assessed. In order to provide an opinion on the risks to health related to the presence of inorganic arsenic in foodstuffs, it was necessary to convert from the arsenic concentrations in drinking-water to total dietary exposure to inorganic arsenic. This conversion required assumptions about the arsenic exposure from food before cooking and the volumes of drinking-water consumed directly and in cooking for the populations in which the respective

health end-points were studied. Because of the uncertainty about actual exposure, the Committee used average estimates of exposure from food and volumes of water consumed to extrapolate from concentrations in drinking-water to total dietary exposure to inorganic arsenic from food and water. A range of low to high values for exposure from food and volume of water consumed was identified to be used in a sensitivity analysis, taking into account the dietary habits and levels of arsenic in food in the relevant region (north-eastern Taiwan, China). The identified ranges were 50–200 µg/day from food excluding water and volumes of 2–4 litres of water consumed directly and used in cooking per day. The average estimates were 75 µg/day from food and 3 litres of water per day. From the available data, an average body weight of 55 kg was assumed for this population.

In order to utilize the adjustment made for other variables (e.g. smoking) in the original analyses in the studies in north-eastern Taiwan, China, of cancers of the lung (*4*) and urinary tract (*5*), adjusted cases were calculated based on the relative risks. This two-step process involved calculating case frequency by multiplying the rate in the referent group by the relative risk and then estimating the number of adjusted cases by multiplying the number of subjects by the case frequency. The resulting adjustment was small relative to the reported number of cases.

In the dose–response analysis using the USEPA BMD software (BMDS version 2.1.1), the nine different dichotomous models were fitted to the adjusted data. Those resulting in acceptable fits based on statistical considerations were selected to derive BMD and BMDL values for a BMR at the low end of the observed range of the data (Table 8). All nine models resulted in an acceptable fit for the lung and urinary tract data. In modelling the epidemiological data, the BMD and BMDL estimated by the log-probit model differed from those of other models, with higher values when the model was constrained within the BMDS and very much lower values when unconstrained. In consequence, the Committee decided that the outputs of the log-probit model should be excluded from the assessment.

Table 8
Ranges of BMD and BMDL values for lung and urinary cancer associated with dietary exposure to inorganic arsenic, based on average estimates of exposure

	$BMD_{0.5}$ (µg/kg bw per day)	$BMDL_{0.5}$ (µg/kg bw per day)
Lung cancer (*4*)	4.5–7.3	3.0–5.0
Urinary tract cancer (*5*)	7.9–13.9	5.2–11.4

$BMD_{0.5}$, benchmark dose for 0.5% increased incidence of cancer over background in north-eastern Taiwan, China, with average 11.5 years of follow-up; $BMDL_{0.5}$, lower 95% confidence limit for the benchmark dose for 0.5% increased incidence of cancer over background.

The lowest calculated BMDL was 3.0 μg/kg bw per day for a 0.5% increased incidence of lung cancer above background over the average 11.5 years of follow-up, based on average estimates of the exposure. A sensitivity analysis to investigate the impact of uncertainty in the exposure estimate in this study indicated that this $BMDL_{0.5}$ could be in the range of 2.0–7.0 μg/kg bw per day, with the assumption made with respect to volume of drinking-water consumed and used in cooking having a greater impact than the assumption regarding inorganic arsenic in food.

Evaluation

From epidemiological studies measuring arsenic levels in drinking-water, inorganic arsenic has been identified as a human carcinogen. It is present naturally in food and water because of geochemical conditions, and consequently exposure varies significantly in different regions and even within regions, primarily through the presence or absence of arsenic in groundwater sources for drinking-water.

The approach to quantitative assessment of cancer risk from inorganic arsenic is limited, inter alia, by the lack of information on total exposure in the available epidemiological studies. The inorganic arsenic BMDL for a 0.5% increased incidence of lung cancer was determined by using a range of assumptions to estimate exposure from drinking-water and food with differing concentrations of inorganic arsenic. The $BMDL_{0.5}$ was computed to be 3.0 μg/kg bw per day (2.0–7.0 μg/kg bw per day based on the range of estimated total dietary exposure). The uncertainties in this $BMDL_{0.5}$ relate to the assumptions regarding total exposure and to extrapolation of the $BMDL_{0.5}$ to other populations due to the influence of nutritional status, such as low protein intake, and other lifestyle factors on the effects observed in the studied population. The Committee noted that the PTWI of 15 μg/kg bw (2.1 μg/kg bw per day) is in the region of the $BMDL_{0.5}$ and therefore was no longer appropriate, and the Committee withdrew the previous PTWI.

The Committee noted that more accurate information on the inorganic arsenic content of foods as they are consumed is needed to improve assessments of dietary exposures to inorganic arsenic species. Analytical constraints to achieving this goal include the lack of validated methods for selective determination of inorganic arsenic species in food matrices and the lack of certified reference materials for inorganic arsenic in foods. The proportion of inorganic arsenic in some foods was found to vary widely, indicating that dietary exposures to inorganic arsenic should be based on actual data rather than using generalized conversion factors from total arsenic measurements.

Reported mean dietary exposure to inorganic arsenic in the USA and various European and Asian countries ranged from 0.1 to 3.0 μg/kg bw per day.

Drinking-water was a major contributor to total inorganic arsenic dietary exposures and, depending on the concentration, can also be an important source of arsenic in food through food preparation and possibly irrigation of crops, particularly rice. The proportion of total exposure to inorganic arsenic arising from food relative to the proportion from water increases as the concentration of inorganic arsenic in the water decreases. At the lower end of the exposure range, food can also be a major contributor to total inorganic arsenic exposure.

For certain regions of the world where concentrations of inorganic arsenic in drinking-water exceed 50–100 µg/l, some epidemiological studies provide evidence of adverse effects. There are other areas where arsenic concentrations in water are elevated (e.g. above the WHO guideline value of 10 µg/l) but are less than 50 µg/l. In these circumstances, there is a possibility that adverse effects could occur as a result of exposure to inorganic arsenic from water and food, but these would be at a low incidence that would be difficult to detect in epidemiological studies.

A detailed addendum to the monograph was prepared.

Recommendations

There is a need for validated methods for selective extraction and determination of inorganic arsenic in food matrices and for certified reference materials for inorganic arsenic.

There is a need for improved data on occurrence of different species of arsenic in, and their bioavailability from, different foods as consumed in order to improve the estimates of dietary and systemic exposure. Further information on the toxicity of arsenic species found in food is also required.

The Committee recommended that future epidemiological studies of the health impacts of arsenic should incorporate appropriate measures of total exposure to inorganic arsenic, including from food and from water used in cooking and processing of food.

Further, it is recommended that epidemiological studies not only focus on relative risks, but also analyse and report the data such that they are suitable for estimating exposure levels associated with additional (lifetime) risks, so as to make their results usable for quantitative risk assessment.

3.3 Deoxynivalenol

Explanation

Deoxynivalenol (12,13-epoxy-3,4,15-trihydroxy-trichothec-9-en-8-one; DON, also known as vomitoxin; CAS No. 51481-10-8) is a type B trichothecene mycotoxin produced mainly in cereals by various *Fusarium*

species. In addition to DON, 3-acetyl-deoxynivalenol (3-Ac-DON; CAS No. 50722-38-8) and 15-acetyl-deoxynivalenol (15-Ac-DON; CAS No. 88337-96-6) are also naturally occurring fungal secondary metabolites, whereas DON-3-glucoside is a naturally occurring conjugate of DON formed in plants.

DON was previously evaluated by the fifty-sixth meeting of the Committee (Annex 1, reference *152*). The Committee established a provisional maximum tolerable daily intake (PMTDI) of 1 µg/kg bw on the basis of the no-observed-effect level (NOEL)[1] of 100 µg/kg bw per day for decreased body weight gain reported in a 2-year feeding study in mice and application of a safety factor of 100. The Committee concluded that intake at this level would not result in effects of DON on the immune system, growth or reproduction. The Committee noted that the available data did not suggest that DON presents a carcinogenic hazard.

DON was on the agenda of the present meeting at the request of the Second Session of CCCF (*6*), which asked the Committee to assess exposure on a more global basis, taking new data into account; to review the toxicological data and consider the need for an acute reference dose (ARfD), taking into account data in finished products, but also in raw wheat and other commodities as they are traded internationally, and consideration of processing factors; and to assess the toxicity of 3-Ac-DON and 15-Ac-DON.

The Committee reviewed several new studies on metabolism and toxicokinetics, acute toxicity, genotoxicity, mechanisms of toxicity and developmental toxicity of DON, including its acetyl derivatives. The Committee also took note of the data previously evaluated at the fifty-sixth meeting. Emphasis was given to studies in which pure DON or acetylated DON was added to defined diets in mammalian species, because naturally contaminated feed commonly contains multiple mycotoxin contaminants. Also, new information on occurrence, processing, prevention and control, and dietary exposure was considered.

Absorption, distribution, metabolism and excretion

The additional studies on metabolism in mice, rats and pigs confirmed that DON and its acetyl derivatives are rapidly and extensively absorbed from the upper gastrointestinal tract and cleared with a short plasma half-life. After absorption of 3-Ac-DON, DON was the principal metabolite observed in plasma, and acetylated DON was not detected, indicating that deacetylation is an extensive and rapid metabolic process. De-epoxidation of DON is a microbial pathway that occurs in the lower gut and does not appear to be a significant route of detoxification in the pig and other monogastric animals.

[1] At the sixty-eighth meeting of the Committee (Annex 1, reference *187*), JECFA decided to differentiate between NOAEL and NOEL. This NOEL would now be considered a NOAEL.

The Committee noted that the new studies on absorption, distribution, metabolism and excretion (ADME) addressed the request made at the fifty-sixth meeting for data from comparative studies on toxicokinetics.

Toxicological data

As concluded at the previous meeting, emesis is the most sensitive functional manifestation of acute toxicity in the pig, dog and cat after either oral or parenteral administration. This is a systemic effect and is believed to arise from increased central serotonergic activity. The lowest doses that did not induce emesis in the pig were 0.025 mg/kg bw by gavage and 0.25 mg/kg bw by exposure via the diet. The Committee took note of the fact that much higher doses were tolerated when DON was given in the diet than by gavage.

New toxicological studies in mice, rats and pigs have provided insights into the mode of action of DON in causing reduced weight gain, which was the basis for the PMTDI established at the fifth-sixth meeting, and into its immunological and related effects in single-dose and repeated-dose studies. These studies indicated that the effects were largely due to the induction of suppressors of cytokine signalling and to effects on the pituitary growth hormone axis. Changes in these parameters are observable very soon after acute dosing in vivo and are rapidly reversible in parallel with the decline in DON concentrations in plasma. At the levels likely to be encountered in the diet (described below), sustained exposure would be necessary to cause functional effects on growth or the immune system.

At its previous evaluation, the Committee concluded that DON is not mutagenic in bacteria but gave rise to chromosomal aberrations both in vitro and in vivo, but their overall significance remained equivocal. The limited new information regarding the potential genotoxicity of DON did not alter the Committee's previous conclusion.

Despite the request from the fifty-sixth meeting of the Committee, no new long-term study in a species other than mouse has become available, and the support for the lack of carcinogenic potential in humans remains dependent on a single mouse study.

One study on reproductive toxicity in rats became available, from which a NOAEL of 1 mg/kg bw per day was derived for reduced epididymal and seminal vesicle weights in rats, as well as increased sperm swimming speed. An additional developmental toxicity study in rats was available in which the NOAELs were 0.5 mg/kg bw per day for maternal toxicity, 1 mg/kg bw per day for fetal toxicity and 2.5 mg/kg bw per day for teratogenicity.

Results from studies on immunotoxicity in mice and pigs showed that low doses of DON increase immunoglobulin A (IgA) levels in the blood. The Committee noted, however, that there were insufficient data with which to

establish a threshold for IgA nephropathy. Most mechanistic studies on immunological end-points in mice and pigs were unsuitable for deriving a NOAEL, but in one study, an acute NOEL of 0.1 mg/kg bw was derived based on suppression of hepatic mRNA for insulin-like growth factor acid-labile subunit. However, the toxicological significance of this finding is unknown.

The Committee considered the toxicity data on derivatives of DON. A few new studies have been published on the toxicity of acetylated DON, and these were considered together with the derivative studies in the previous evaluation. Given the results from the ADME studies, the toxicity of the acetylated DON compounds is likely to arise from conversion to DON. In vitro cytotoxicity and immunotoxicity studies of the relative potencies of DON and its acetylated derivatives are not considered to provide a reliable indication of relative potency in vivo, as they generally do not take account of this conversion. Median lethal dose (LD_{50}) studies indicated their toxicity in mouse to be similar to that of DON. The acetylated DON compounds were therefore considered to be as toxic as DON.

No toxicological studies were found on DON-3-glucoside, a fungal metabolite recently detected in wheat and beer. The Committee considered it possible that this compound would be hydrolysed in the body and the DON would become bioavailable, but noted that ADME studies would be necessary to confirm this.

Observations in humans

No new epidemiological studies were found. With respect to possibilities for derivation of a NOAEL from outbreaks of mycotoxicosis in humans, recent studies indicate that urinary biomarkers may be used for assessing human exposure to DON. As DON can be formed from its acetylated derivatives, the Committee considered that these biomarkers could provide an indication of total dietary exposure to DON and its derivatives. Using the limited information on outbreaks from epidemiological studies summarized for the previous evaluation, the Committee noted that the calculated level that was not likely to elicit acute intoxication in humans was around 50 µg/kg bw.

Analytical methods

The Committee reviewed the range of screening and quantitative methods available for the determination of DON in various foods after the fifty-sixth meeting and noted that a number of advances have been made in the analysis of both DON and its derivatives and that certified standard solutions for DON, 3-Ac-DON and 15-Ac-DON have been made available.

Immunoassays for screening purposes for DON have been further developed and, in some instances, commercialized. These methods include lateral flow devices, fluorescence polarization and direct fluorometry after extract cleanup and derivatization. New antibodies continue to be developed. Possible cross-reactivity between DON and its derivatives in enzyme-linked immunosorbent assays (ELISA) has been demonstrated in comparative studies and possibly accounts for the previously noted higher levels of naturally occurring DON determined by ELISA as opposed to chromatographic methods. Commercialized screening methods are usually developed with LODs targeted to meet legislative or other requirements.

Major advances have been made in DON determination by HPLC in which analytical methods using ultraviolet (UV) detection for DON in cereals (oat flour, wheat flour and rice flour), cereal products (polenta and wheat-based breakfast cereals), soft wheat and baby food have been validated by international collaborative studies. These methods, using either immunoaffinity column or multifunctional column cleanup, have been validated down to 60 µg/kg for baby foods and to 100 µg/kg for all other products. The application of HPLC coupled to MS has enabled multi-mycotoxin analysis to be undertaken. The major problem of LC-MS and LC-MS/MS—namely, matrix effects in which signal enhancement or suppression occurs—is generally overcome by the use of isotope-labelled internal standards or matrix-matched standard solutions. These methods can be used for a limited range of mycotoxins for which a common cleanup, such as by multi-mycotoxin immunoaffinity column, is available; alternatively, a more diverse analysis can be performed by an injection of an aliquot of diluted sample extract without prior cleanup.

Based on current knowledge, the main derivatives of DON that might contribute to exposure are 3-Ac-DON, 15-Ac-DON and DON-3-glucoside. The analysis of these compounds requires chromatographic separation. They can be determined simultaneously with DON by LC-MS/MS. Alternatively, the acetyl derivatives have been determined by GC after suitable derivatization.

Sampling protocols

Owing to the lack of homogeneity in the distribution of mycotoxins, the sampling stage of the overall mycotoxin analysis can frequently represent the greatest contribution to the overall variance of the result. This was noted by the fifty-sixth meeting of the Committee. Specific sampling protocols for DON should be followed, such as the one provided by the European Commission, which regulates the number and size of incremental samples as well as the size of the aggregate sample to be taken for control purposes.

Effects of processing

The fifty-sixth meeting of the Committee reviewed the effects of gravity separation, milling, washing, soaking in water or sodium carbonate solutions, baking, extrusion cooking, fermentation and the use of microorganisms on DON levels. These are documented in the monograph of the fifty-sixth meeting of the Committee (Annex 1, reference *153*). Milling redistributes DON, with the highest amounts appearing in the bran, which is sometimes used in human food and most often in animal feed. Additional studies conducted since then have shown that removal of screenings and bran from wheat grains reduced DON levels by 41–50%. Current data have also confirmed the efficacy of washing or soaking in water or sodium carbonate solutions in reducing DON levels in barley and wheat. Although results of frying and baking studies have been conflicting, the use of extrusion cooking indicated a reduction of DON levels by between 18% and 95%, depending on the moisture content and temperature. It is, however, suggested that apparent reductions may be due to binding or the inability to extract the toxin from the extruded matrix using current analytical techniques. Few studies exist on the effects of malting and brewing processes on DON levels. Steeping lowered DON levels as a result of the water solubility of the toxin. During germination, DON levels increased 2-fold because of the conditions conducive for *Fusarium* growth and toxin formation. A subsequent decrease in DON levels during fermentation was observed, which was attributed to yeast absorption. Additional studies are required to confirm these changes as well as the effects of processing on the acetyl derivatives of DON.

Prevention and control

Prevention and control practices include the use of suitable crop rotation, appropriate use of fertilizers, irrigation and weed control, and the use of resistant cultivars and decontamination procedures. The use of microorganisms is a recent approach employed to reduce growth of *Fusarium* species, severity of disease symptoms and DON levels. Strains of *Bacillus subtilis*, *Fusarium equiseti* and *Cryptococcus* sp. have given encouraging results (controlling *Fusarium* head blight and reducing DON formation) in field studies with wheat. Experimental studies under glasshouse conditions with *Streptomyces* sp., *Pseudomonas fluorescens* and *Pseudomonas frederiksbergensis* strains similarly reduced both the severity of *Fusarium* head blight symptoms caused by *Fusarium culmorum* in wheat and barley and DON levels under both glasshouse and field conditions. The use of chitosan (deacetylated derivative of chitin) for reducing DON levels as well as the severity of *Fusarium* head blight symptom development in wheat and barley has been studied, but additional data are required to confirm the effects.

No new data are available on the use of chemicals such as sodium bisulfite, hypochlorite bleach, ammonia, moist ozone, and natural and modified clays to decontaminate grain.

Levels and patterns of contamination in food commodities

Information on the occurrence of DON was drawn from data received from a number of countries (Austria, Belgium, Brazil, China, Finland, France, Hungary, Japan, the Netherlands, Norway, Singapore and the United Kingdom), surveys published in the open literature from 42 countries, as well as the European Commission's Scientific Co-operation on Questions relating to Food (SCOOP) report on mycotoxins. Only DON data published since the previous evaluation were included in this assessment. In total, data on 23 980 samples analysed for DON were collected (68% from Europe, 17% from Asia, 6% from North America, 5% from South America and 3% from Africa). It was noted that DON remains a common contaminant in cereals (wheat, maize, oats, rye, barley, rice) and their products. Highest reported mean levels for raw cereals were as follows: wheat, 9900 µg/kg; maize, 4772 µg/kg; rice, 183 µg/kg; barley, 6349 µg/kg; oats, 537 µg/kg; and rye, 190 µg/kg. Contamination levels vary widely between and within regions. Relatively lower levels were detected in processed products, such as baby food, beer, bread, biscuits, pasta, muesli, noodles, cereal-based snacks, pizza, polenta, couscous, flours and fermented soya bean, most likely due to the decrease in contamination resulting from cereal milling and processing. Mean levels of DON in samples of processed products did not exceed 1250 µg/kg. As noted by the fifty-sixth meeting of the Committee, carry-over of DON into animal products is negligible due to feed refusal, rapid metabolism and elimination in livestock species.

The occurrence data for the DON derivatives 3-Ac-DON and 15-Ac-DON in wheat, maize, barley, oats, rye and their products were considered by the Committee for the first time at the present meeting. In addition to data submitted by China, France, Japan and the United Kingdom, published data from studies conducted in nine countries were also assessed. Data were available on 3-Ac-DON from 6980 samples (92% from Europe and 8% from Asia) and on 15-Ac-DON from 4300 samples (81% from Europe, 16% from Asia and 3% from the USA). Generally, these derivatives are infrequently detected, and levels were typically less than 10% of those reported for DON. Highest reported mean levels in wheat, maize and barley for 3-Ac-DON were 193 µg/kg, 27 µg/kg and 19 µg/kg, respectively; for 15-Ac-DON, the corresponding highest reported mean levels were 365 µg/kg, 236 µg/kg and 0.3 µg/kg. The Committee was aware of reports on DON-3-glucoside in cereals and beer (data on 500 samples were assessed, with 79% from China,

15% from Europe and 6% from the USA), but considered that the data were too limited for dietary exposure assessment.

Food consumption and dietary exposure assessment

Dietary exposure to DON was evaluated at the fifty-sixth meeting of the Committee. Using the then-available five regional diets from GEMS/Food, the total dietary exposure to DON was estimated to range from 0.77 μg/kg bw per day in the African diet to 2.4 μg/kg bw per day in the Middle Eastern diet. The major source of dietary exposure in three of the five regional diets (European, Latin American and Middle Eastern) was wheat (64–88% of total exposure), whereas the sources in the other two regional diets were more varied (wheat, rice and maize in the African diet and wheat and rice in the Far Eastern diet).

At the current meeting, the Committee prepared updated international estimates using the consumption cluster diets from GEMS/Food and occurrence data reported in the literature or supplied to the Committee by countries. Information was available on the concentrations of DON in six commodities: barley, maize, oats, rice, rye and wheat. Additionally, information on beer, the majority of which is produced from barley, was included. Data originating in 42 countries were analysed, representing 10 of the 13 GEMS/Food consumption cluster diets; no data were reported for the A, H and J clusters. Of the six commodities for which information was available for the exposure assessment, data on DON concentrations in barley, maize and wheat predominated, with limited reports on concentrations in oats, rice and rye. In total, 401 data points (mean values) representing 16 569 individual samples sorted by specific cluster diet were included in the exposure assessment. As the acetylated derivatives of DON are, in general, found at levels less than 10% of those for DON, they were not included in the dietary exposure estimates. Their inclusion would not be expected to change the estimates significantly.

The average dietary exposures to DON were calculated by multiplying the weighted mean concentration of each commodity by the corresponding amount of each commodity consumed in each of the 10 GEMS/Food consumption cluster diets for which occurrence data were available. The total dietary exposure to DON was estimated to range from 0.2 μg/kg bw per day (cluster C) to 14.5 μg/kg bw per day (cluster B). The main source of exposure in clusters C, D, E, F, G, K, L and M was wheat (56–100% of total exposure), whereas the main source in clusters B and I was maize. Three of the clusters had dietary exposure estimates above the PMTDI of 1 μg/kg bw established previously. The Committee noted that the high estimates of dietary exposure to DON in clusters B and M were due to unusually high reported DON levels

in maize and wheat in single countries for each cluster and that these data may not be representative of chronic dietary exposures. The range of estimates in the remaining clusters is in agreement with those prepared at the fifty-sixth meeting. It should be noted that any reduction in the concentration of DON as a result of processing has not been taken into consideration in this assessment.

Since the evaluation of DON at the fifty-sixth meeting of the Committee, a number of national evaluations of dietary exposure to DON have been published. The Committee considered evaluations of dietary exposure to DON from Argentina, Belgium, Czech Republic, Denmark, Ethiopia, France, Germany, Ireland, Japan, Lebanon, Morocco, the Netherlands, Nigeria, Republic of Korea and Thailand. Some of these reports contained overall dietary exposure assessments, whereas others assessed single commodities (or their products) considered to be the potential primary source of dietary exposure to DON in the population assessed. The evaluations that contained numerical estimates are summarized in Table 9.

For risk characterization, the Committee chose a dietary exposure of 0.5 µg/kg bw per day for an average exposure and 1.0 µg/kg bw per day for a high exposure.

The Committee was asked to consider the need for an ARfD for DON. In this regard, the Committee prepared an estimate of acute dietary exposure to DON. The Committee chose to use a high-percentile daily consumption (97.5th, taken from the WHO GEMS/Food database) with a high concentration of DON (and its acetyl derivatives) in food (the highest mean value taken from the review of occurrence data at the present meeting). The consumptions for the foods most likely to be contaminated with DON were as follows: maize, 4.06 g/kg bw per day; wheat flour, 9.17 g/kg bw per day; white bread, 9.08 g/kg bw per day; and wheat, 13.46 g/kg bw per day. Considering that breads were the mostly likely foods to be regularly consumed, the Committee used a figure of 9 g/kg bw per day in making the estimate. Combining this with a DON contamination level of 10 mg/kg of wheat gives an acute dietary exposure estimate of 90 µg/kg bw per day. The Committee noted that regulatory limits for DON in foods in various countries range up to 1 mg/kg food. Using this limit with the high consumption figure would result in an acute dietary exposure of 9 µg/kg bw per day.

Dose–response analysis

The Committee was aware that acute exposure to high doses of DON and its derivatives has resulted in emesis in humans and considered it appropriate to establish an ARfD. Although developmental toxicity might be considered a potential effect of acute intoxication during critical periods of embryogenesis,

Table 9
National dietary exposures to DON[a]

Country	Mean exposure (μg/kg bw per day)	Upper-percentile exposure (μg/kg bw per day)
Argentina	0.02–0.06 (breads)	Not reported
Belgium	<0.07 (beer)	0.23 (97.5th, beer) 0.05 (eggs)
Czech Republic	Not reported	3 (4–19 years, 99th)
Denmark	0.02–0.03 (adults) 0.32 (children)	2 (4–19 years, 99th) 0.9 (children, 99.9th)
France	0.28 (adults) 0.45 (children) 0.32–0.45 (vegetarians)	0.57 (adults, 95th) 0.93 (children, 95th) 0.72–0.96 (vegetarians, 95th)
Germany	0.45 (adults) 0.19 (children)	0.90 (adults) 0.38 (children)
Ireland	0.001 (milk)	0.02 (milk)
Japan	Not reported	0.69 (1–6 years, 95th) 0.49 (7–14+ years, 95th) 0.24 (>19 years, 95th)
Lebanon	0.55 (8–13 years) 0.41 (14–18 years)	<1.0 (8–13 years, 95th) 0.66 (14–18 years, 95th)
Netherlands	0.46 (1 year) 0.66 (children) 0.2 (10+ years)	1 (4–19 years, 99th) 1 (1 year, 95th) 0.4 (10 years, 95th)
Republic of Korea	0.1 (7+ years) 0.14 (3–6 years)	0.2 (7+ years) 0.30 (3–6 years)

[a] Where no age group is specified, the dietary exposure is for the total population; when a food is specified, the dietary exposure included consumption of that food only.

the NOAEL for teratogenicity in the rat was 1 order of magnitude greater than the level found not to induce emesis in the pig; therefore, emesis in pigs was chosen to derive an acute health-based guidance value. Because the emetic effect was considered to be dependent on the maximum plasma concentration (C_{max}), the Committee concluded that for the purpose of establishing an ARfD, studies in which DON was administered via the diet were more appropriate than studies that used gavage dosing.

Data on DON-induced emesis in pigs, cats and dogs were available; although the effect was noted at similar concentrations in the three species, the dog and cat data were deemed not suitable for dose–response modelling. Two studies on emesis in piglets and pigs following exposure to DON via the diet (*7*, *8*) were combined for BMD modelling. Doses were calculated from the measured DON concentrations in the feed and the observed feed intake. In the first study, dietary concentrations above 3 mg/kg of feed resulted in drastically reduced average feed intakes (reduced by 88–94% compared with controls) and decreases in body weights during the test period; for these

groups, it was assumed that the total feed intake over 4 or 11 days was actually all consumed on day 1. This assumption was made because it has often been observed that pigs stop eating after DON-induced vomiting on day 1. For the three dose groups in which it was reported that at least one pig vomited, it was assumed that the incidence was one. In the second study, the average feed intake was taken from the first week of exposure, although intake was decreased in the dose groups given 1.4 mg/kg of feed or more compared with controls. The initial body weights were used for the calculations, because emesis was observed on day 1 of exposure.

The dose–response analysis was performed using the PROAST software (version 23.2). The BMR was set at 10% extra risk. The $BMDL_{10}$s among the accepted models ranged between 0.21 and 0.74 mg/kg bw per day. The lowest value in this range was used as a POD for establishing an ARfD.

Evaluation

At its fifty-sixth meeting, the Committee established a PMTDI of 1 µg/kg bw for DON on the basis of the NOEL[1] of 100 µg/kg bw per day based on decreased body weight gain from a 2-year feeding study in mice and application of a safety factor of 100. Repeated-dose short-term studies considered in the present evaluation indicated that this NO(A)EL remains appropriate.

Since 3-Ac-DON is converted to DON and therefore contributes to the total DON-induced toxicity, the Committee decided to convert the PMTDI for DON to a group PTMDI of 1 µg/kg bw for DON and its acetylated derivatives (3-Ac-DON and 15-Ac-DON). In this regard, the Committee considered the toxicity of the acetylated derivatives equal to that of DON. The Committee concluded that, at this time, there was insufficient information to include DON-3-glucoside in the group PMTDI.

The Committee derived a group ARfD for DON and its acetylated derivatives using the lowest $BMDL_{10}$ of 0.21 mg/kg bw per day for emesis in pigs. The Committee considered that because DON-induced emesis is a systemic effect and more dependent on C_{max} than on area under the plasma concentration–time curve (AUC), it would be appropriate to apply an uncertainty factor of 25, which is the value used by the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) for acute C_{max}-dependent effects. The Committee established a group ARfD for DON and its acetylated derivatives of 8 µg/kg bw. Limited data from human case reports indicated that dietary exposures to DON up to 50 µg/kg bw per day are not likely to induce emesis.

[1] At its sixty-eighth meeting (Annex 1, reference *187*), the Committee decided to differentiate between NOAEL and NOEL. This NOEL would now be considered a NOAEL.

Estimation of dietary exposure was made using data from 42 countries, representing 10 of the 13 GEMS/Food consumption cluster diets, and was therefore considered to be more globally representative than the previous evaluation. The Committee concluded that all of the mean estimates of national exposure to DON were below the group PMTDI of 1 µg/kg bw. National reports showed dietary exposures that were above 1 µg/kg bw per day in only a few cases, only for children at upper percentiles. For acute dietary exposure, the estimate of 9 µg/kg bw per day, based on high consumption of bread and a regulatory limit for DON of 1 mg/kg food, was close to the group ARfD.

The acetylated derivatives have not been included in the estimates of dietary exposure to DON prepared at this meeting. The Committee noted that in general they are found at levels less than 10% of those for DON, and inclusion would not be expected to significantly change the estimates of dietary exposure to DON. Data are limited on the occurrence of DON-3-glucoside, which might be an important contributor to dietary exposure; this derivative was also not included in the dietary exposure estimates.

A detailed addendum to the monograph was prepared.

Recommendations

- As DON-3-glucoside has been detected in cereals and beers and might therefore contribute to systemic exposure to DON, the Committee recommended that ADME studies be conducted on this substance.
- Additional data on the occurrence of and the effects of processing on 3-Ac-DON, 15-Ac-DON and DON-3-glucoside are needed, as well as their co-occurrence with DON.

3.4 Furan

Explanation

Furan (C_4H_4O) (CAS No. 110-00-9) is a highly volatile cyclic ether that can be formed unintentionally in foods during processing from precursors that are natural food components. Information available to the Committee at its present meeting suggested that the major route of exposure to furan in the human population is through consumption of heat-treated foods and beverages.

Furan has not been evaluated previously by the Committee. The request for a full evaluation of furan originated from the Second Session of CCCF (*6*).

Absorption, distribution, metabolism and excretion

Following oral administration to mice and rats, furan is rapidly absorbed, metabolized and eliminated in urine and faeces as metabolites and exhaled in air as unchanged furan and carbon dioxide formed as a result of ring opening. The initial ring-opened metabolite is *cis*-2-butene-1,4-dial (BDA), which is formed in the liver in a reaction catalysed by CYP2E1. Furan-derived products are most abundant in the liver of dosed animals. A variety of identified urinary metabolites could arise from amino acid or protein crosslinking.

Toxicological data

The toxicity of orally administered furan has been extensively studied in mice and rats over a wide dose range. The primary site of toxicity of furan is the liver, although the kidneys and lungs are also affected at high doses (>30 mg/kg bw per day). In addition, changes in some haematological and hormonal parameters occur at doses as low as 0.12 mg/kg bw per day administered 5 days/week.

Regarding hepatotoxicity, uncoupling of hepatocyte mitochrondrial oxidative phosphorylation is an early critical event in cytolethality. Liver cell injury, including oxidative stress, progresses to cell death. This, in turn, gives rise to regenerative responses, including increased hepatocellular proliferation in mice and rats and, notably in the rat, an early proliferative reaction involving the biliary epithelium, referred to as cholangiofibrosis. These proliferative changes may be the basis for liver tumorigenicity, either alone or in combination with DNA alteration. Although furan is not genotoxic in a number of test systems and binding to rat liver DNA was not detectable, the metabolite BDA is highly reactive and binds to proteins and nucleic acids. BDA produced DNA strand breaks in cultured mammalian cells and was mutagenic in bacteria and cultured mammalian cells; being a dialdehyde, it also formed crosslinks with DNA of cultured cells. The in vitro genotoxicity of BDA allows the possibility that BDA formed in vivo from furan could react with DNA.

Several cancer bioassays of orally (gavage) administered furan in mice and rats have been performed. In mice, doses of 8 and 15 mg/kg bw per day, 5 days/week (*9*), and 0.5, 1.0, 2.0, 4.0 and 8.0 mg/kg bw per day, 5 days/week (*10*), were used. In rats, doses of 2, 4 and 8 mg/kg bw per day, 5 days/week, were administered (*9*). In livers of male and female rats, high incidences of cholangiocarcinomas were induced at all doses in the NTP study (*9*), accompanied by biliary tract hyperplasia, metaplasia and fibrosis. Hepatocellular neoplasms were increased at lower incidences. In both sexes of rat, furan also increased the incidences of mononuclear cell leukaemia, albeit against unusually low background incidences in control groups. In male and female mice in both studies, only hepatocellular neoplasms were increased.

Observations in humans

No epidemiological studies were available.

Analytical methods

GC-MS has been shown to be the most suitable technique for the reliable detection of low levels of furan in foods. GC-MS is usually preceded by headspace (HS) extraction or headspace solid-phase microextraction (HS-SPME). Both HS and HS-SPME approaches are simple and convenient and give satisfactory results for analyses of volatiles. Owing to the high volatility of furan, food samples and standards need to be chilled and handled quickly. Puréed, liquid samples or reconstituted powdered samples can be transferred directly to HS vials, whereas solid samples have to be homogenized. Most published methods include the use of deuterium-labelled furan as an internal standard, which is normally added to the homogenized sample before the extraction. LODs and limits of quantification (LOQs) from 0.1 to 5 ng/g and from 0.4 to 13 ng/g, respectively, have been reported for methods based on HS extraction. Lower LODs and LOQs are reported for methods using HS-SPME. No certified reference material is currently available.

Formation, effect of processing and fate in foods

Furan can be formed in a variety of foods from different precursors by thermal and non-thermal processing (ionizing radiation). The proposed routes for furan formation are mainly based on 1) Maillard reactions, 2) thermal degradation of carbohydrates, 3) thermal degradation of certain amino acids, 4) thermal oxidation of ascorbic acid, polyunsaturated fatty acids and carotenoids and 5) free radical reactions during irradiation. Higher amounts of furan are normally formed under roasting conditions (dry heating, 200 °C, 10 min) compared with pressure cooking conditions (sterilization, 121 °C, 25 min), and pH plays a complex role in the mechanism of furan formation. For coffee, the amount of furan formed in beans varies according to the level of roasting. Grinding may reduce furan levels by 10–60%, and further decreases occur in the production of instant coffee powder and in brewing.

Limited data are available on the formation of furan in home-cooked food as well as on the stability of furan during cooking, storing and reheating of meals. As furan appears to be well dissolved within the matrix, opening the jars (e.g. baby foods) exposes only a relatively small surface area. Therefore, despite furan's volatility, its evaporation is hindered by its slow diffusion inside the food matrix. However, if canned or jarred foods are heated in a saucepan under stirring, larger declines of furan content can be observed. Studies on the losses of furan during warming procedures for ready-to-eat foods have shown conflicting results, with some authors reporting losses of

29–85% and others finding that furan persists during normal heating practices. Losses of furan in heated foods left for cooling seem to be insignificant.

Levels and patterns of contamination in food commodities

Furan concentration data covering 21 countries were submitted by Australia, Brazil, Canada, the European Union (EU), Japan, Republic of Korea, Switzerland and the USA. The total number of analytical results (single or composite samples) evaluated at the present meeting was 5662, with 59.8% from Europe, 16.7% from North America, 22.8% from Asia, 1.0% from Latin America and 0.7% from the Pacific region. The occurrence of furan has been investigated mainly in thermally processed foods, such as coffee, canned and jarred foods, including baby foods, soups and sauces. The ranges of national mean levels of furan for the foods with the highest contamination levels were as follows: roasted coffee (powder), 814–4590 μg/kg; instant coffee (powder), 90–783 μg/kg; brewed roasted coffee, 34–113 μg/kg; jarred baby foods, 19–96 μg/kg; soya sauce, 16–52 μg/kg; canned fish, 6–76 μg/kg; and baked beans, 27–581 μg/kg. Lower levels have been found in other foods, including products from vegetables, meat, milk and cereals.

Food consumption and dietary exposure assessment

Although the presence of furan as a flavour component in food was first reported in 1979, dietary exposure assessments for furan were not undertaken until 2004, when data on furan concentrations in a variety of foods in the USA became available.

At the present meeting, the Committee considered dietary exposure estimates for furan submitted by the USA, the EU and Brazil, all of which were based on analysed data for foods and individual dietary records for the populations of interest. The dietary exposure estimates for the whole population, infants and young children in the USA and Denmark were considered by the Committee to underestimate dietary exposure, as furan levels were assigned to the specific foods analysed only and hence did not represent the whole food supply. In contrast, the dietary exposure estimates for adults submitted by the EU for 14 European countries were considered to be overestimates; as the mean furan levels from 2004–2009 results for individual foods were grouped and then assigned to the food consumption amount for the relevant wider food group, as described in the EFSA Concise European Food Consumption Database, some uncertainty was introduced in these dietary exposure assessments. For example, the furan level for coffee was assigned to the wider food group “coffee, tea and cocoa”; as levels of furan are much higher in coffee than in either tea or cocoa, this results in an overestimate of dietary exposure to furan from these beverages.

For infants and young children, concern has been expressed about potential dietary exposure to furan from the consumption of baby foods sold in jars or cans. Estimates of dietary exposure to furan for infants in Europe and Brazil assumed that all food consumed by the infants had been in jars or cans; these estimates were considered appropriate for infants fed solely on these products, but would be overestimates of dietary exposure for the whole infant population.

As furan occurs primarily in heat-processed foods, the Committee noted that international estimates using the GEMS/Food consumption cluster diets could not be generated, as appropriate food consumption data were not available for heat-processed foods.

In general, mean dietary exposure to furan from national assessments ranged from 0.25 to 1.17 μg/kg bw per day for adults, from 0.08 to 0.23 μg/kg bw per day for children 1–6 years of age and from 0.27 to 1.01 μg/kg bw per day for infants up to 12 months of age. For consumers at high percentiles of dietary exposure, estimates ranged from 0.60 to 2.22 μg/kg bw per day for adults and from 0.99 to 1.34 μg/kg bw per day for infants; no high-percentile dietary exposure data were available for children. Estimates of dietary exposure to furan are summarized in Table 10.

Table 10
Estimates of dietary exposure to furan

Country	Dietary exposure estimate (μg/kg bw per day)	
	Mean	Upper percentile
Europe		
Europe[a]	0.29–1.17 adults	0.60–2.22 adults (95th)
	0.27–1.01 infants 3–12 months	1.14–1.34 infants 6–9 months (95th)
Denmark[b]	0.95–1.02 adults	2.10–2.19 adults (95th)
	0.08 children 4–6 years	
North America		
USA[c]	0.25–0.26 adults	0.61 adults (90th)
	0.23 children 2–5 years	
	0.41 infants 0–12 months	0.99 infants 0–12 months (90th)
South America		
Brazil[d]	0.46 infants 6–11 months	1.34 infants 6–11 months (99th)

[a] Individual dietary records for 14 European countries from the Concise European Food Consumption Database; analysed furan values from period 2004–2009.
[b] Individual dietary records from the Danish National Nutrition Survey; new furan data for some heat-processed foods; EFSA data for other foods.
[c] Individual dietary records from the USA 1994–1996, 1998 supplementary CSFII; analysed furan values from 2003 and 2007 surveys.
[d] Individual dietary records for infants; analysed data for baby food.

For adults, coffee was the major contributor to dietary furan exposures (40–80%), with cereals, vegetables, meats and dairy foods contributing more than 5% to total exposure. For children, breakfast cereals were the major contributor (40%). As reported furan levels were much higher for brewed coffee than for ready-to-drink instant coffee, the type of coffee consumed in a given population and the furan level for coffee influenced the dietary exposure estimates for adults. The lower values obtained in the United States Food and Drug Administration (USFDA) estimates for adults compared with those from EFSA for European countries were largely explained by the lower furan level for brewed coffee in the USFDA estimates. Despite this, estimated dietary furan exposures available to the Committee were in the same order of magnitude.

For the purposes of risk characterization, a value of 1 μg/kg bw per day was taken to represent mean dietary exposure to furan, and a value of 2 μg/kg bw per day was taken to represent high dietary exposure. The Committee considered these values to be sufficient to cover potential dietary exposures of infants and children to furan.

Dose–response analysis

Dosing with furan in bioassays gave rise to increases in liver tumours and leukaemias. The neoplasms evaluated for dose–response analysis were as follows: cholangiocarcinomas in livers of male and female rats, hepatocellular neoplasms in male and female rats, mononuclear cell leukaemias in male and female rats and hepatocellular neoplasms in male and female mice.

The cholangiocarcinomas were seen only in rats and were associated with extreme hepatotoxicity and an early and marked biliary tract proliferative response. The relevance for humans of the cholangiocarcinomas is not clear, and the available data do not allow for an analysis of the mode of action. Also, the high incidences of these neoplasms at all doses of furan precluded identification of a POD. The Committee was aware of ongoing studies in rats to extend the dose–response data and address mechanistic aspects for this end-point.

The mononuclear cell leukaemias in rats, which occur in high incidence in the strain used in the NTP bioassay (*9*), are of unknown pathogenesis, and the increases occurred against a background of unusually low incidences in the control groups. Moreover, studies of genotoxicity in rat bone marrow, where the progenitor cells of the leukaemias presumably arise, were negative, and the mode of action is unknown.

The hepatocellular neoplasms in rats in the NTP study (*9*) and in mice in the NTP study (*9*) and the study of Moser et al. (*10*) and the leukaemias in rats in the NTP study (*9*) were selected for modelling.

In the dose–response analysis using the USEPA BMD software (BMDS version 2.0), the nine different statistical models were fitted to the experimental data considered relevant for further consideration. Those resulting in acceptable fits based on statistical considerations (chi-squared test, $P > 0.1$) were selected to derive the BMD and BMDL for a 10% extra risk of tumours. This procedure resulted in a range of BMD_{10} and $BMDL_{10}$ values for each end-point considered (Table 11).

The BMD_{10}s and $BMDL_{10}$s derived from the different data were broadly similar. Those for the hepatocellular adenomas and carcinomas in male mice were the lowest but varied over a broad range, and there was a high incidence of liver tumours in the control male mice. The study of Moser et al. (*10*) had more and lower doses and a greater number of animals in the low-dose group compared with the NTP studies (*9*). For each study, a comparison of the BMD_{10}s and $BMDL_{10}$s derived with the dosages used indicates that those derived from the Moser et al. study (*10*) are much closer to the dosage levels used in that study (*9*). This indicates that the BMD_{10}s and $BMDL_{10}$s derived from the Moser et al. study (*10*) had less uncertainty than those derived from the NTP studies (*9*). Therefore, the Committee decided to use the $BMDL_{10}$ of 1.34 mg/kg bw per day, which corresponds to 0.96 mg/kg bw per day when adjusted from a 5 days/week dosing schedule to an average daily dose, in female mice derived from the hepatocellular adenoma and carcinoma data from the Moser et al. study (*10*) as the POD.

Table 11
BMD_{10} and $BMDL_{10}$ values for tumours associated with administration of furan by gavage[1]

Tumour	Study	Sex and species	BMD_{10} (mg/kg bw per day)[a]	$BMDL_{10}$ (mg/kg bw per day)[a]
Hepatocellular adenomas and carcinomas	Moser et al. (*10*)	Female mice	1.87–2.86	1.34–1.89
	NTP (*9*)	Male mice	0.49–6.66	0.35–1.85
	NTP (*9*)	Female mice	1.63–6.88	1.07–4.20
	NTP (*9*)	Male rat	1.64–1.92	1.00–1.34
	NTP (*9*)	Female rat	4.82–6.47	3.16–5.25
Leukaemias	NTP (*9*)	Male rat	1.66–2.47	0.97–1.98
	NTP (*9*)	Female rat	2.13–2.98	1.18–2.29

BMD, benchmark dose for 10% extra risk of tumours; BMDL, 95% lower confidence limit for the benchmark dose. Extra risk is defined as the additional incidence divided by the tumour-free fraction of the population in the controls.

[a] BMD_{10}s and $BMDL_{10}$s have not been adjusted for the dosing schedule of 5 days/week.

Evaluation

MOEs were calculated at dietary exposures of 0.001 mg/kg bw per day, to represent the average dietary exposure to furan for the general population, and 0.002 mg/kg bw per day, to represent the dietary exposure to furan for consumers with high exposure. This estimate will also cover dietary exposure of children. Comparison of these dietary exposures with the $BMDL_{10}$ of 0.96 mg/kg bw per day for induction of hepatocellular adenomas and carcinomas in female mice gives MOEs of 960 and 480 for average and high dietary exposures, respectively. The Committee considered that these MOEs indicate a human health concern for a carcinogenic compound that might act via a DNA-reactive genotoxic metabolite.

The furan levels can be reduced in some foods through volatilization (e.g. by heating and stirring canned or jarred foods in an open saucepan). However, there is currently a lack of quantitative data for all foods, and no information is available on other mitigation methods.

A detailed monograph was prepared.

3.5 Mercury

Explanation

Mercury occurs naturally in the earth's crust, usually in the form of the mineral cinnabar (mercury(II) sulfide). It can be released into the global environment through a number of processes, both natural and anthropogenic. While relatively chemically inert, mercury occurs in three valence states: elemental mercury (also known as metallic mercury), monovalent mercurous ion and divalent mercuric ion, elemental mercury and the divalent ion being the most important in nature. There are several organic mercury compounds; by far the most common in the environment and in the aquatic food-chain is methylmercury.

Mercury was previously evaluated by the Committee at its tenth, fourteenth, sixteenth and twenty-second meetings (Annex 1, references *13*, *22*, *30* and *47*). At its sixteenth meeting, the Committee allocated a PTWI of 0.3 mg of total mercury (5 µg/kg bw), of which no more than 0.2 mg (3.3 µg/kg bw) should be in the form of methylmercury, based primarily on the relationship between the intake of mercury from fish and mercury levels in blood and hair associated with the onset of clinical disease. The sixteenth meeting of the Committee noted that almost all dietary exposure to methylmercury is from fish and seafood and that methylmercury is probably by far the most toxic form of mercury in food; therefore, other forms of mercury could be given less weight when establishing a tolerable intake for mercury. The original PTWI for methylmercury (3.3 µg/kg bw) was revised at the sixty-first

meeting (Annex 1, reference *166*) to 1.6 µg/kg bw, based on an assessment of results from various epidemiological studies involving fish-eating populations and developmental neurotoxicity. At the sixty-seventh meeting (Annex 1, reference *184*), the Committee provided further clarifications as to the relevance of the new methylmercury PTWI for different subgroups of the population.

At the sixty-first meeting, the Committee recommended that the total mercury PTWI be reviewed.

Absorption, distribution, metabolism and excretion

Following oral exposure, inorganic mercury salts show limited absorption, which is related to their water solubility. In human volunteers, the average absorption of a tracer dose of inorganic mercury given as mercury(II) nitrate was 5–10%, whether delivered in a protein-bound matrix or as a solution.

Inorganic mercury compounds are not lipid soluble and do not readily cross the blood–brain barrier or placenta membranes. Ionic species of inorganic mercury readily bind to sulfhydryl groups of various thiol-containing compounds, such as glutathione, cysteine and metallothionein. Kidneys exhibit the greatest concentration of mercury following exposure to inorganic mercury compounds. The main pathways of excretion of absorbed inorganic mercury are via the urine and, to a lesser extent, in the faeces. Owing to the poor absorption of orally administered inorganic mercury, the majority of the ingested dose in humans is excreted in the faeces. Inorganic mercury can also be excreted via the breast milk. The half-life for inorganic forms of mercury in humans has been estimated at 1–2 months.

Toxicological data

Haematological, hepatic and renal effects have been reported in rats or mice administered sublethal single oral doses of mercury(II) chloride. Renal effects usually observed with mercury(II) chloride at doses above 5 mg/kg bw per day include interstitial sclerosis, renal tubular damage and proximal tubular necrosis. Severe gastrointestinal damage, including inflammation and necrosis of the forestomach and necrosis of the glandular stomach, can also be induced with high doses of inorganic mercury, in particular for mercury(II) compounds, which are relatively more corrosive than mercury(I) compounds.

Longer-term exposure (subchronic to chronic) to inorganic mercury at doses above 1–5 mg/kg bw per day can induce a variety of effects related to general toxicity (decrease in body weight gain, changes in clinical and haematological parameters), as well as organ-specific effects (increased kidney and adrenal weights, testicular atrophy). Effects associated with relative kidney weight changes include marked thickening of glomerular and tubular basement

membranes, degeneration and atrophy of the tubular epithelium and increased severity of nephropathy. Treatment of mice and rats by gavage with mercury(II) chloride at doses ranging from 1.25 to 20 mg/kg bw per day and from 0.312 to 5.0 mg/kg bw per day, respectively, for 6 months produced a variety of renal effects, which occurred with greater frequency and severity in male animals. Unlike organic mercury compounds, neurotoxicity is not usually observed, even at exposure levels that produce frank toxicological effects in other organs.

Reproductive effects induced by inorganic mercury include decreased fertility, reduced implantation efficiency and decreases in both live births and litter sizes. The observed effects seem to involve male-specific end-points (testicular atrophy, androgen decreases, spermatogenesis disruption) more than effects in females. However, inconsistencies have been noted in some experimental responses. A consistent observation in most reproduction studies includes increased relative kidney weights in the offspring.

Inorganic mercury compounds have produced some genotoxic effects in vitro and in vivo, with stronger evidence from in vitro experiments, including single-stranded DNA breaks, sister chromatid exchanges and chromosomal aberrations. However, the mechanisms appear to involve primarily induction of oxidative stress (reactive oxygen species) or disruption of microtubules rather than direct interaction with DNA, including adduct formation, which has not been demonstrated.

Chronic exposure of mice and rats to mercury(II) chloride at doses ranging from 2.5 to 10 mg/kg bw per day has produced some indications of carcinogenicity. The main findings included an increased incidence of forestomach hyperplasia, forestomach squamous cell papillomas and a marginal increase in thyroid follicular cell carcinomas in male rats. In mice, renal tubule tumours were seen only in high-dose males, but the incidence was not statistically significant compared with historical controls. It was concluded by the NTP (*11*) that there was some evidence of carcinogenic activity of mercury(II) chloride in male F344 rats, based on the increased incidences of squamous cell papillomas of the forestomach and the marginally increased incidence of thyroid follicular cell neoplasias, equivocal evidence in both female rats and male mice, and no evidence in female rats. However, the NTP (*11*) considered that the forestomach lesions in male rats may have limited relevance, as they did not progress to malignancy (direct tissue irritation effect). Also, as follicular cell carcinomas in rats usually result from increased incidences of hyperplasia and adenomas, it was further noted that the combined incidence of thyroid follicular cell neoplasms (adenomas and carcinomas) was not significantly increased. IARC considered that there is limited evidence in experimental animals for the carcinogenicity of mercury(II) chloride, based on results from the NTP (*11*) bioassay.

Observations in humans

Human data on the adverse health effects of exposure to inorganic mercury, including renal effects, consist of case reports or case series that do not allow the identification of dose–response relationships. Therefore, they do not provide an adequate basis for deriving a health-based guidance value. They do, however, provide evidence that supports the use of adverse renal effects observed in experimental species as the basis for such a derivation. Nephrotic syndrome, including proliferative or membranous glomerulonephritis, has been associated with the topical use of mercury(II) ammonium chloride creams. Based on the limited number of studies of cancer and the absence of consistent findings, IARC concluded that there is inadequate evidence in humans for the carcinogenicity of mercury and mercury compounds. As a result, inorganic mercury compounds were not classifiable as to their carcinogenicity in humans (Group 3).

Analytical methods

Sample handling is generally critical only for water samples. The best materials for water sample storage and processing are polytetrafluoroethylene and fluorinated ethylene-propylene. Fresh samples are usually stored deep-frozen, lyophilized in darkness or sometimes sterilized. It has been reported that methylmercury may be decomposed in some food matrices with repeated freezing and unfreezing (particularly in bivalves). However, relatively little is known about the effect of storage on the stability of methylmercury in food samples.

Following acidic digestion of samples, cold vapour atomic absorption spectrometry (CV-AAS) or cold vapour atomic fluorescence spectrometry (CV-AFS) has been widely used for the determination of total mercury in several food matrices. An LOQ of about 30 μg/kg dry mass in foods may be obtained by CV-AAS. Further sensitivity enhancement may be obtained by CV-AFS. The main advantages of the cold vapour technique are the separation of the analyte from the potentially interfering sample matrix and its comparatively low cost. However, to avoid interference by CV-AFS, special precautions must be taken to completely remove vapours when nitric acid is used for digestion. With an LOQ of about 10 μg/kg dry mass and greater selectivity, ICP-MS is increasingly being used with an addition of gold chloride to mercury standard solutions to avoid the mercury memory effects. Although the instrumentation is expensive to purchase and to operate, the ability of ICP-MS to provide low LOQs, to provide a wide dynamic linear range and to measure many elements simultaneously can offset these cost factors.

Basically, all the speciation methodology is generally targeted on the separation and determination of methylmercury, and there has been no conclusive identification of other species of mercury.

Extraction of the mercury species from its matrix requires an aggressive treatment, such as acid digestion, distillation or alkaline extraction, with the option of applying ultrasonic or microwave energy to assist in the procedure. Extraction is one of the most critical steps, because two conflicting issues need to be addressed: obtaining high extraction efficiency and preventing losses. In alkaline media, methylmercury appears to be more stable than in acid media, with proteins being easily hydrolysed.

GC has been the most widely used technique for the separation of mercury species, whereas HPLC is increasingly being applied. The detection methods (LOD in parentheses) of CV-AAS (10 μg/kg), CV-AFS (1 μg/kg), microwave-induced plasma (MIP)-AES or ICP-AES (5 μg/kg), MS (40 μg/kg) and ICP-MS (<3 μg/kg) all have sufficient sensitivity for food samples. The advantage of MS and ICP-MS is their multielement and multiisotope capabilities that allow for more accurate and precise results by speciated isotope dilution (SID)-MS, which can also check for species transformations and extraction recoveries. Once in solution, methylmercury may decompose when exposed to light, low pH and high storage temperatures. Other factors, such as the type of storage container, may also affect the stability.

Available certified reference materials and proficiency testing schemes or intercomparison exercises exist for both total mercury and methylmercury to demonstrate and maintain analytical quality assurance. However, there is a current need for fully validated, standardized methods for determination of methylmercury and inorganic mercury.

Sampling protocols

Some authorities have regulations with regards to specific sampling protocols for mercury and other contaminants. For example, the European Commission has regulated the number and size of incremental samples, size of the aggregate sample and precautions to be taken for control purposes.

Levels and patterns of contamination in food commodities

At its present meeting, the Committee reviewed data from eight countries on the occurrence of mercury in different food commodities analysed between 1997 and 2009. The total number of analytical results for total mercury was more than 106 740, with 93% coming from Europe (Finland, France, Spain), 5% from Asia (Japan, China), 1% from the Americas (Brazil, Canada) and 1% from Oceania (Australia), for water (85%), fish (6%), shellfish (2%) and other food groups (6%). The 2128 samples analysed for methylmercury were

from fish (94%), shellfish (2%) and other products (4%). However, the Committee did not receive any occurrence data on inorganic mercury in foods or water.

Total mercury levels in 98% of 90 545 water samples analysed in France were below the LOQ of 0.02 µg/l, with a maximum of 4.3 µg/l.

Total mercury levels in foods other than fish products were generally low (range 0.0001–0.050 mg/kg), with about 80% of the 6183 samples containing levels below the LOQs. The highest levels were found in fungi. Mean methylmercury levels reported by China in non-fish samples ranged from 0.001 to 0.023 mg/kg, with a maximum concentration found in poultry. No other information on methylmercury in non-fish samples was received from other countries.

Total mercury levels in 1892 shellfish samples (80% above LOQ) ranged from 0.002 to 0.86 mg/kg. No shellfish species contained methylmercury at concentrations greater than 0.5 mg/kg (range 0.002–0.451 mg/kg), with the maximum concentration found in edible crab.

Total mercury levels in 6114 fish samples ranged from 0.001 to 11.4 mg/kg, with the maximum concentration found in marlin.

The proportion of total mercury contributed by methylmercury generally ranged between 30% and 100%, depending on species of fish, size, age and diet. Furthermore, in about 80% of these data, methylmercury accounted for more than 80% of total mercury. However, a few submitted data showed proportions of methylmercury of about 10% or less.

Food consumption and dietary exposure assessment

National estimates

Most of the available dietary exposure assessments for mercury were from national TDSs. These include the following TDSs: Australia (2000–2001), Canada (1998–2000), China (2007), Czech Republic (2000), France (2001–2002), Japan (2008), New Zealand (2003–2004), Republic of Korea (2005), the United Kingdom (2006) and the USA (1991–2005). Published data from other studies focusing on special subpopulations were also available. These include TDSs conducted in Chile (Santiago) and Spain (Catalonia) and studies of fishermen and their household members in Zhoushan Island (China), residents of Changchun city in north-east China, secondary-school students in Hong Kong Special Administrative Region, frequent seafood consumers in France (Fish and Seafood Consumption Study and Biomarkers of Exposure to Trace Elements, Pollutants and Omega-3, or the CALIPSO study), exposures from fish and shellfish in Spain and modelled exposure estimates for fish consumers in the USA.

In general, most studies available allowed for the estimation of dietary exposure to total mercury from fish and shellfish as well as from other foods. Table 12 summarizes the estimates of mean dietary exposure to total mercury from the total diet, from fish and shellfish, and from other foods extracted from the studies listed above. Estimated mean dietary exposure to total mercury ranged from 0.07 to 5.81 µg/kg bw per week, while the estimated mean dietary exposure to total mercury from fish and shellfish ranged from 0.07 to 1.75 µg/kg bw per week. The estimated mean dietary exposure to total mercury from foods other than fish and shellfish ranged from 0 to 4.06 µg/kg bw per week. The upper limit of that range corresponds to a subpopulation of children. When only total population or subpopulations of adults were considered, the estimated mean dietary exposure to total mercury from foods other than fish and shellfish ranged from <0.01 to 1.01 µg/kg bw per week. The main contributors to this average dietary exposure were breads and cereals.

The studies did not provide 90th-percentile estimates of the dietary exposure to total mercury from foods other than fish and shellfish; hence, the 90th-percentile exposure estimates were derived by multiplying the mean exposure estimates by 2. The resulting 90th-percentile exposure to total mercury from foods other than fish and shellfish was estimated to range from <0.02 to 2.03 µg/kg bw per week for the general population or adult subpopulations and from <0.02 to 8.12 µg/kg bw per week when children subpopulations are included.

The contribution of fish and shellfish to the total dietary exposure ranged from 40% to 100% when samples with non-detectable concentrations were assigned a zero concentration. Estimates of per cent contribution to total dietary exposure for foods other than fish and shellfish based on dietary exposure estimates derived from concentration data using the LOR or LOR/2 for non-detects are not reliable because they artificially inflate the contribution of these foods, particularly when the LOR is high. Only studies from which it was possible to separately estimate the contribution of fish and shellfish and other foods to total dietary exposure to mercury are presented in Table 12.

It was assumed that the predominant source of inorganic mercury in the diet is foods other than fish and shellfish.

International estimates

The available mercury occurrence data were deemed to be not sufficiently representative for use in deriving international estimates of dietary exposures in combination with food consumption from the GEMS/Food consumption cluster diets. No international estimates of dietary exposure were prepared.

Table 12
Contribution of fish and shellfish to dietary exposure to total mercury (national estimates)

Country	Average dietary exposure to total mercury (µg/kg bw per day)			% from fish and shellfish
	Total diet	Fish and shellfish	Other foods	
Estimates derived by assigning a zero value to samples with concentrations below the LOD				
Australia TDS	0.01–0.02	0.01–0.02	0–0	100–100
Canada TDS (excluding infants)	0.01–0.03	0.01–0.02	<0.01–0.02	51–80
Chile (Santiago)	0.06	0.02	0.03	41
China (Zhoushan Island)	0.47–0.92	0.41–0.87	0.05–0.10	87–95
Japan TDS	0.17	0.16	0.01	92
Republic of Korea TDS	0.04	0.03	0.01	76
United Kingdom TDS	0.02–0.04[a]	—	—	—
USA TDS	0.01–0.02	0.01–0.02	<0.01–<0.01	96–100
Estimates derived by assigning a non-zero value (LOD or LOQ) to samples with concentrations below the LOD or LOQ				
Australia TDS	0.08–0.26	0.01–0.02	0.06–0.24	7–17
Canada TDS (excluding infants)	0.01–0.04	<0.01–0.04	0.01–0.03	40–74
Chile (Santiago)	0.08	0.02	0.06	31
United Kingdom TDS	0.04–0.12[b]	—	—	25
Estimates derived by assigning a non-zero value (LOD/2 or LOQ/2) to samples with concentrations below the LOD or LOQ				
China TDS	0.08	0.01	0.07	13
China (Changchun city)	0.10	0.01	0.09	13
France TDS	0.16–0.26[c]	0.02–0.02	0.15–0.24	9–10
New Zealand TDS	0.11–0.16	0.08–0.10	0.03–0.06	65–74
Spain (Catalonia)	0.28–0.83	0.12–0.25	0.14–0.58	30–46
Range (µg/kg bw per day)[d]	**0.01–0.83**	**0.01–0.25**	**0–0.58**	**—**
Range (µg/kg bw per week)[d]	**0.07–5.81**	**0.07–1.75**	**0–4.06**	**—**

[a] High exposures (97.5th percentile) ranged from 0.07 to 0.17 µg/kg bw per day.
[b] High exposures (97.5th percentile) ranged from 0.12 to 0.26 µg/kg bw per day.
[c] High exposures (95th percentile) ranged from 0.25 to 0.41 µg/kg bw per day.
[d] Excluding the study of fishermen and their families in Zhoushan Island, China.

Dose–response analysis

Kidney effects are consistently observed in various experimental species (weight changes, proximal tubule damage and progressive nephropathy). Relative kidney weight increases observed in rats following exposure to mercury(II) chloride are also associated with a dose-dependent increase in renal mercury accumulation and with significant changes in the renal

cortex, including increases in both proximal tubule and glomerular volumes. The Committee therefore considered it appropriate to model kidney weight changes, which generally occurred at doses similar to or lower than other renal effects. Data on relative kidney weight increases were taken from the NTP study (*11*), in which rats and mice of both sexes were exposed by gavage to mercury(II) chloride, 5 days/week for 6 months. Other end-points from this study were considered (i.e. terminal body weight, serum alkaline phosphatase and cholinesterase, incidence of mild nephropathy) for BMD modelling (data not shown); however, the BMDLs generated were greater than those estimated for increased relative kidney weight. Models that passed the goodness of fit test ($P > 0.10$) were considered to be acceptable, and the lowest BMDL was selected from these models (Table 13). The 6-month exposure was deemed sufficient to establish a health-based guidance value because the half-life of mercury(II) chloride in rats is estimated at less than 30 days, steady-state renal mercury concentrations were reached by 4–6 months and exposures in the same dose range for longer durations produced early mortality. The Committee further considered that a 10% change for increased relative kidney weight was appropriate as a BMR to establish a health-based guidance value. This decision was based on the following: the kidney weight data were modelled based on reported mean values, animals in the lowest experimental dose group (0.325 mg/kg bw per day) already exhibited a 10% increase in mean relative kidney weight and the severity of nephropathy was significantly increased only at doses greater than or equal to 1.25 mg/kg bw per day.

Evaluation

The Committee noted that there was a lack of quantitative data on methylmercury in non-fish products and on inorganic mercury in general.

The Committee assumed that the predominant form of mercury in foods other than fish and shellfish is inorganic mercury. While data on speciation of inorganic mercury in foods are limited, the Committee agreed that the toxicological database for mercury(II) chloride was relevant for assessing the health

Table 13
Dose–response modelling[a] for a 10% increase in relative kidney weight for male and female F344 rats gavaged with mercury(II) chloride for 6 months[b]

Sex	BMD_{10} (mg/kg bw per day as mercury(II) chloride)	$BMDL_{10}$ (mg/kg bw per day as mercury(II) chloride)
Males	0.22–0.31	0.11–0.18
Females	0.43–0.45	0.19–0.25

[a] BMDS version 2.1.1.
[b] BMD(L)s have not been adjusted for the dosing schedule of 5 days/week.

risk of foodborne inorganic mercury. The results of the NTP bioassay (*11*) provided limited evidence for carcinogenicity; however, direct reaction of mercury(II) chloride with DNA has not been demonstrated. Therefore, setting a health-based guidance value was considered appropriate.

The lowest $BMDL_{10}$ for relative kidney weight increase in male rats was calculated to be 0.11 mg/kg bw per day as mercury(II) chloride. This corresponds to 0.06 mg/kg bw per day as mercury, adjusted from a 5 days/week dosing schedule to an average daily dose and for the per cent contribution of inorganic mercury to mercury(II) chloride dose. After application of a 100-fold uncertainty factor, the Committee established a PTWI for inorganic mercury of 4 µg/kg bw (rounded to one significant number).

The previous PTWI of 5 µg/kg bw for total mercury, established at the sixteenth meeting, was withdrawn.

In the absence of evidence to the contrary, the new PTWI for inorganic mercury was considered applicable to dietary exposure to total mercury from foods other than fish and shellfish. The upper limits of estimates of average dietary exposure to total mercury from foods other than fish and shellfish for adults (1 µg/kg bw per week) and for children (4 µg/kg bw per week) were at or below the PTWI.

A detailed addendum to the monograph was prepared.

Recommendations

There is a need for:

- validated analytical methods for both inorganic mercury and methylmercury applicable in several food matrices;
- more information on the inorganic mercury and methylmercury content of foods as consumed that mainly contribute to overall dietary exposure.

3.6 Perchlorate

Explanation

The perchlorate ion (ClO_4^-) is very stable in water, and its salts are highly soluble in water. Perchlorate occurs naturally in the environment, in deposits of nitrate and potash, and can be formed in the atmosphere and precipitate into soil and groundwater. It also occurs as an environmental contaminant arising from the use of nitrate fertilizers and from the manufacture, use and disposal of ammonium perchlorate (CAS No. 7790-98-9) used in rocket propellants, explosives, fireworks, flares and air-bag inflators and in other industrial processes. Perchlorate can also be formed during the degradation

of sodium hypochlorite used to disinfect water and can contaminate the water supply. Water, soil and fertilizers are considered to be potential sources of perchlorate contamination in food. Potassium perchlorate (CAS No. 7778-74-7) has been used as a human therapeutic medicine to treat thyroid disease.

Perchlorate has not been previously evaluated by the Committee. It was referred to the Committee for evaluation on request of the Second Session of CCCF (*6*).

Absorption, distribution, metabolism and excretion

Perchlorate is rapidly absorbed following ingestion and rapidly excreted unchanged, mostly in the urine. It crosses the placenta and is also found in breast milk.

Relevance of animal data for human risk assessment

The health effects of perchlorate salts are due to the perchlorate ion itself. In the studies described below, doses are expressed in terms of perchlorate ion. The primary effect of perchlorate is its ability to competitively inhibit uptake of iodide by the thyroid gland. The inhibition is at the level of the sodium–iodide symporter (NIS), which actively transports iodide and perchlorate from the blood into the thyroid gland. Inhibition of iodide uptake by perchlorate reduces the amount of iodide available for the synthesis of thyroid hormones, resulting in reductions in the concentrations of circulating thyroxine (T_4) and the more biologically active hormone, triiodothyronine (T_3). In a negative feedback loop, reductions in the concentrations of T_4 and T_3 reaching the brain trigger the release of thyrotropin-releasing hormone in the hypothalamus, which, in turn, causes the release of thyrotropin, also known as thyroid stimulating hormone (TSH), from the anterior pituitary gland. TSH initiates the events in the thyroid that result in NIS transport of iodide into the thyroid and synthesis of T_3 and T_4. Sustained reduction in iodide uptake by the thyroid may result in hypothyroidism. Hypothyroidism has adverse implications for structural and functional brain development in the fetus, infant and child and for metabolism and the functioning of cardiovascular, gastrointestinal, skeletal, neuromuscular and reproductive systems in adults.

The Committee noted that for substances known to affect the thyroid and having a mode of action involving inhibition of the uptake of iodide, the rat is not a good model for humans. This applies not only to the likelihood of thyroid cancer but also to other perturbations of thyroid physiology and pathology in response to thyroid toxicants. For this reason, the animal data reviewed by the Committee, which comprised mostly data from rats and were qualitatively supportive of the human data, were not further considered for deriving a health-based guidance value for perchlorate.

Observations in humans

Human observations include clinical studies on thyroid function in healthy adult volunteers given perchlorate in drinking-water, clinical studies on thyroid function in workers occupationally exposed to perchlorate for several years, population-based epidemiological studies and ecological studies comparing populations living in areas with differing concentrations of perchlorate in the drinking-water. Some of the ecological studies included pregnant women, newborns and children and, in the case of newborns, took advantage of neonatal screening programmes that included measurements of serum TSH and/or thyroid hormones. In the various studies, outcome measures investigated included one or more of the following: radiolabelled iodide uptake by the thyroid, serum TSH and thyroid hormone concentrations, urinary perchlorate and iodide concentrations, incidence of thyroid diseases, neonatal birth weight, head circumference and length, incidence of congenital hypothyroidism and neurobehavioural measures in children. There are also historical clinical data from past use of perchlorate as a treatment for hyperthyroidism.

None of the ecological studies showed any relationship between perchlorate concentrations in drinking-water and the incidence of thyroid diseases, including congenital hypothyroidism and thyroid cancer. With respect to TSH and thyroid hormones, one study reported a significant association between elevated newborn TSH levels and concentrations of perchlorate in drinking-water. However, it is notable that the concentrations of perchlorate in drinking-water in this study were not as high as in other, negative studies on newborns.

The clinical studies in healthy adult volunteers and workers did not show any significant effects on TSH or thyroid hormone concentrations at daily exposures of up to 0.5 mg/kg bw per day. From consideration of human clinical studies in healthy subjects and studies of long-term treatment of patients with hyperthyroidism, the National Academy of Sciences (NAS) in the USA has estimated that a sustained exposure to perchlorate of more than 0.4 mg/kg bw per day would probably be necessary in order to trigger hypothyroidism in normal adults. The NAS also commented that in pregnant women, infants, children and people with low iodide intake or pre-existing thyroid dysfunction, the dose of perchlorate required to cause hypothyroidism may be lower. Despite lack of effects on TSH and thyroid hormones, the studies in healthy adult volunteers did show clear, dose-related effects on radiolabelled iodide uptake by the thyroid.

A key issue for human risk assessment is whether a POD for deriving a health-based guidance value for perchlorate should be based on the end-point of inhibition of thyroidal uptake of iodide or on end-point(s) with clearer

implications for adversity, such as increases in TSH, reductions in circulating thyroid hormone levels and clinical hypothyroidism. Inhibition of iodide uptake by the thyroid is clearly a precursor event in the chain of events that ultimately can lead to adverse effects on thyroid function; by itself, however, it cannot be considered adverse if circulating thyroid hormone levels remain unchanged. The available human data provide support for such a conclusion. For example, for short-term exposure, the 14-day clinical study by Greer and co-workers (*12*) in healthy adults found no effect on TSH or thyroid hormones at the highest perchlorate dose tested, 0.5 mg/kg bw per day, despite the observation that the highest dose caused an average 67% inhibition of iodide uptake by the thyroid. For chronic exposure, support is provided by data from the two occupational studies, in which about half the participants in each study had been exposed for more than 5 years. In one study, perchlorate exposure equivalent to about 0.5 mg/kg bw per day (the same as the highest dose in the Greer et al. study (*12*)) was not associated with any change in TSH or thyroid hormones; in the other study, in which uptake of radiolabelled iodide by the thyroid was measured, a 38% reduction in iodide uptake was not associated with any effect on TSH or thyroid hormones. The NAS review also expressed the view that uptake of iodide by the thyroid would need to be inhibited by at least 75% for several months or longer in adults with a normal dietary iodine intake in order to cause declines in thyroid hormone production that would have adverse health effects. A further practical constraint in selecting the end-point is that, with the exception of one study, the human clinical and epidemiological studies on perchlorate did not actually identify any significant association between perchlorate exposure and changes in TSH and thyroid hormones that could be used for the POD.

A second key issue is to what extent a POD derived from studies in healthy adults relates to potentially more vulnerable groups in the population. Given the critical role of thyroid hormones in brain development, it is widely considered that the probability of a permanent adverse effect on neurodevelopment from thyroid disruption, including transient disruption, would be greatest during early life. Consideration needs to be given to the differing thyroid physiology in the fetus and neonate compared with that of children and adults; for example, the amount of thyroid hormone stored in the colloid in late-gestation fetuses and neonates is estimated to be sufficient for less than 1 day only, compared with several months for adults. It is also unclear whether nursing infants may be additionally at risk if perchlorate were to reduce the passage of iodide into breast milk; at present, there are very few data on this, and what data are available are contradictory.

As perchlorate competitively inhibits iodide uptake by the thyroid, another issue for consideration is whether populations living in parts of the world where the diet is deficient in iodine would be more susceptible to perchlorate than iodine-replete individuals. The data on this aspect are sparse. A further

consideration is whether individuals, in particular pregnant women, who already have the condition of hypothyroidism or subclinical hypothyroidism might be additionally affected by low-level perchlorate exposure. The above subgroups of the general population are numerically significant.

The Committee also noted that there can be co-exposure in the diet to other ubiquitous anti-thyroid substances with the same mode of action (i.e. competitive inhibition at the NIS), such as nitrate or thiocyanate, or with differing modes of action, such as organochlorines.

Given the mode of action of perchlorate, the key vulnerable groups are likely to be pregnant women, fetuses, newborns, young infants, those with hypothyroidism and possibly those with iodine-deficient diets. As there are no good quantitative data relating perchlorate dietary exposure to changes in thyroidal iodide uptake, TSH or thyroid hormones in these key groups that are comparable with the quantitative data obtained in the study by Greer and co-workers (*12*), these represent significant data gaps and are the major sources of uncertainty in any population-wide risk assessment. The uncertainties discussed above need to be weighed alongside the fact that the POD for the risk assessment can be based on inhibition of iodide uptake by the thyroid, a precursor event that, at least at low to moderate levels of inhibition, appears to be non-adverse.

Analytical methods

Perchlorate is soluble in water and polar organic solvents and is easily extracted from foods using either water or water–acetonitrile mixtures. Water samples are analysed directly, whereas food extracts are subjected to solid-phase extraction (SPE) cleanup prior to determination. Analytical methods used for the detection and determination of perchlorate levels in water and foods include ion chromatography (IC) with conductivity detector, IC-MS or IC-MS/MS and LC-MS or LC-MS/MS. IC-MS/MS and LC-MS/MS methods offer lower detection limits and can be used for the determination of perchlorate in foods. Stable isotope–labelled perchlorates are used as internal standards. Currently, certified reference materials are not available, and collaborative method validation studies have not been conducted. Other methods, such as spectrophotometry, capillary electrophoresis and ion-selective electrode–based potentiometric methods, lack in sensitivity and may not be suitable for detection at lower levels in foods and water. Rapid screening methods have not yet been developed. Most survey data in foods have been obtained using the IC-MS/MS method.

Effects of processing

Limited data are available on the fate of perchlorate during food processing. However, perchlorate is generally stable at temperatures used in food processing.

Prevention and control

Reduction of perchlorate in foods and water relies mainly on the control of contamination in fertilizers, irrigation systems and water used in food processing and food preparation. However, washing can reduce surface contamination in vegetables and fruits.

Levels and patterns of contamination in food commodities

At the present meeting, the Committee reviewed data from six countries on the occurrence of perchlorate in water and different foods analysed between 2004 and 2009 (Table 14). Analytical data on 35 073 samples of groundwater and drinking-water indicate that the perchlorate level in 98% of samples was below the LOR. In drinking-water, perchlorate levels exceeded 20 µg/l in 1% of the samples only. The USEPA reported that only 160 out of 3870 water supplies (4%) had perchlorate levels above the LOR (4 µg/l), in the range of 4–420 µg/l, with a mean concentration of 9.9 µg/l; this mean concentration is the average of the detected concentrations. Analytical data in 1866 samples (vegetables, fruits, rice, milk, infant formula, fish and fish products, and beverages, such as juices, beer and wine) were reviewed, and perchlorate levels in 33% of samples were found to be below the LOR. Weighted mean (mean of reported mean levels weighted by number of samples) perchlorate levels in raw vegetables were in the range of 4.8–110 µg/kg (potato, 4.8 µg/kg; carrot, 6.6 µg/kg; spinach, 110 µg/kg; lettuce, 11.6 µg/kg; tomato, 14 µg/kg; squash, 75 µg/kg; eggplant, 78 µg/kg; broccoli, 19 µg/kg; cauliflower, 7 µg/kg; cabbage, 10 µg/kg); in fruits, the weighted mean levels ranged from 0.5 to 28 µg/kg (oranges, 5 µg/kg; apples, 0.5 µg/kg; grapes, 28 µg/kg; melons, 19 µg/kg). Other weighted mean perchlorate levels were as follows: rice, 1 µg/kg; whole wheat flour, 3.5 µg/kg; milk, 6.8 µg/kg; beer, 1 µg/kg; and wine, 6 µg/kg. The mean perchlorate levels in human milk in China (n = 24 composite samples, with each composite sample representing milk from 50 mothers) and the USA (n = 652) were found to be 19.7 µg/l (range 2.1–136 µg/l) and 9.3 µg/l (range 0.01–411 µg/l), respectively. Limited data show that the weighted mean perchlorate level in infant formula was 10 µg/kg. The Committee noted that sampling and analysis were carried out on targeted foods. However, general surveys involving a broader range of foods in different countries were lacking. In view of the widespread presence of perchlorate in the environment, it is probable that it will be found more widely in drinking-water and food.

Food consumption and dietary exposure assessment

The Committee evaluated occurrence and dietary exposure data for perchlorate from submissions from China, Japan, Canada and the USA and from the literature. International estimates of dietary exposure were prepared using food consumption information from the 13 GEMS/Food consumption cluster diets and perchlorate concentrations discussed above. The range of estimated

Table 14
Summary of perchlorate occurrence data from various countries, 2004–2009

Region	Country	Food		Water	
		Number of analytical results	% of values below LOR	Number of analytical results	% of values below LOR
Asia	China	92	0	83	40
	India	—	—	66	24
	Japan	209	7	50	28
	Republic of Korea	—	—	146	0
North America	Canada	500	26	—	—
	USA	1 065	41	34 728	98
Total		**1 866**	—	**35 073**	—

LOR, limit of reporting (detection or quantification limit)

dietary exposures to perchlorate is 0.03–0.22 µg/kg bw per day for the 13 GEMS/Food consumption cluster diets. Milk consumption accounts for a large portion of the dietary exposure to perchlorate for most clusters, ranging from 7% (clusters C and G) to 42% (cluster J) of the total. These estimates do not include dietary exposure from drinking-water. Using the WHO default drinking-water consumption for adults of 2 litres per day and the mean concentration from the USEPA data of 9.9 µg/l in samples in which perchlorate was detected, the Committee estimated that additional perchlorate exposure from drinking-water could be 20 µg/day (0.33 µg/kg bw per day).

The Committee also reviewed national dietary exposure estimates from Canada and the USA. The Canadian estimates were based on data taken from foods available in a public market using a deterministic approach. Perchlorate concentrations were combined with food consumption data available from the Nutrition Canada Food Consumption Survey (<18 years old) and the Nova Scotia Provincial Nutrition Survey (adults >18 years old, including women of childbearing age). Mean dietary exposures to perchlorate were higher for children than for adults. The mean dietary exposure was approximately 0.04 µg/kg bw per day for children 1–11 years of age compared with 0.03 µg/kg bw per day for adults, including women of childbearing age.

For the population of the USA, an estimate of dietary exposure to perchlorate was made using urinary biomarker concentrations of perchlorate, available from the 2001–2002 National Health and Nutrition Examination Survey. The dietary exposure (which would include exposure from water) was estimated using the ratio of the perchlorate to creatinine concentrations in urine, assuming that 100% of perchlorate in the diet is absorbed and excreted

unmetabolized. The 50th-percentile estimate for the total adult population was 0.06 μg/kg bw per day, whereas the 95th-percentile estimate was 0.23 μg/kg bw per day.

An analysis of perchlorate dietary exposures in the USA from the USFDA's TDS from 2003–2008 was submitted to the Committee. The mean estimates for all age subgroups 14 and over were approximately 0.1 μg/kg bw per day, with 90th-percentile estimates less than 0.23 μg/kg bw per day. Dietary exposure was greatest for 2-year-olds; the mean exposure for this group was 0.44 μg/kg bw per day, with a 90th-percentile exposure of 0.73 μg/kg bw per day.

The Committee concluded that the analyses from the USFDA's TDS provide the best estimates of dietary exposure to perchlorate, as they do not depend on analyses of raw commodities and include water and beverages made from water that might be contaminated with perchlorate. Although all of the national estimates are in general agreement, the Committee noted that the highest estimates were from the TDS, suggesting that the other estimates (and the international estimates prepared by the Committee) may not include all dietary sources of perchlorate.

The highest estimate of dietary exposure from the USFDA's TDS of 0.7 μg/kg bw per day for 2-year-old children and the mean estimate of 0.1 μg/kg bw per day for those 14 years of age and older were chosen for comparison with the health-based guidance value.

Dose–response analysis

The pivotal study for dose–response analysis was the human clinical study on 16 male and 21 female healthy adult volunteers published by Greer and co-workers in 2002 (*12*), in which perchlorate was given in drinking-water at doses of 0.007, 0.02, 0.1 or 0.5 mg/kg bw per day for 14 days. The uptake of iodide into the thyroid was measured at baseline before administration of perchlorate and on days 2 and 14 of administration at both 8 and 24 h after administration of radiolabelled iodine. These data were used for modelling.

The Committee selected a critical effect size of 50% inhibition of iodide uptake as the BMR. This choice was made because human clinical data from healthy adults following both short-term and chronic exposure to perchlorate have shown that such a level of inhibition is not associated with any changes in TSH or thyroid hormone levels. The Committee noted that a BMR of 50% inhibition was within the observed range of the study.

PROAST software (version 23.2) for analysis of continuous data was used. In the clinical study, each subject served as his or her own control, with baseline values measured 1 day before the start of the 14-day perchlorate exposure

period. In the BMD analysis, the baseline value for iodide uptake in each subject was set at 100%. Analysis of the post-treatment values for iodide uptake at 8 and 24 h and the values from exposure days 2 and 14 as covariates showed that these factors did not have any significant impact. Therefore, the two models used for continuous data—the exponential and Hill models—were fitted to all the data combined. Similar values for the BMD_{50} and $BMDL_{50}$ were obtained from the two models, as shown in Table 15. The lower of the two $BMDL_{50}$ values (rounded to two significant figures) of 0.11 mg/kg bw per day was used as the POD.

Evaluation

As perchlorate has a very short half-life and is rapidly cleared from the body, it is considered appropriate to derive a PMTDI. The $BMDL_{50}$ of 0.11 mg/kg bw per day for inhibition of uptake of radiolabelled iodide by the thyroid was chosen as the POD for derivation of a PMTDI. As it is based on human data, there is no need to apply any interspecies uncertainty factor.

The Committee noted that the $BMDL_{50}$ was derived from a study of relatively short duration but that there are efficient homeostatic mechanisms to cope with short-term and long-term inhibition of iodide uptake, up to (at least) 50%, in healthy children and adults. The Committee also noted that there is at least a 4-fold margin between the value of the $BMDL_{50}$ and the estimate of more than 0.4 mg/kg bw per day that would probably be necessary as a sustained exposure in order to trigger hypothyroidism in normal adults. The Committee therefore concluded that it was not necessary to apply an uncertainty factor to account for the short duration of the pivotal study.

In considering the size of any necessary uncertainty factor for inter-individual human differences, the Committee took account of the fact that the effect of perchlorate on inhibition of iodide uptake by the thyroid and on the subsequent synthesis of thyroid hormones in potentially vulnerable groups—such as pregnant women, fetuses, neonates and young infants, those with iodine-deficient diets and those with clinical or subclinical hypothyroidism—may differ from that in healthy adults. The Committee concluded

Table 15
BMD and BMDL values for 50% inhibition of uptake of radiolabelled iodine by the thyroid in humans exposed to perchlorate for 14 days (*12*)

Model	Number of regression parameters	BMD_{50} (mg/kg bw per day)	$BMDL_{50}$ (mg/kg bw per day)
Exponential model	4	0.137	0.114
Hill model	4	0.141	0.117

that an uncertainty factor of 10 would be appropriate to cover any differences in the general population, including those in potentially vulnerable subgroups. Applying this 10-fold factor to the $BMDL_{50}$ and rounding to one significant figure, a PMTDI of 0.01 mg/kg bw was established for perchlorate.

The estimated dietary exposures of 0.7 μg/kg bw per day (highest) and 0.1 μg/kg bw per day (mean), including both food and drinking-water, are well below the PMTDI. The Committee considered that these estimated dietary exposures were not of health concern.

A detailed monograph was prepared.

4. Future work

The Committee recognized that the use of dose–response modelling is a developing field and recommends to the Joint FAO/WHO Secretariat that an expert working group be established to review progress and develop detailed guidance for the application of the methods most suitable to the work of the Committee. The working group should, inter alia, address the following aspects:

- the use of constraints when modelling;
- the weighting of model outcomes and model averaging;
- goodness of fit criteria;
- how human data might be used for dose–response modelling to derive a POD;
- presentation of modelling outcomes in JECFA publications.

5. Recommendations

Exposure

The seventy-second meeting of the Committee evaluated six contaminants occurring in various foods. The Committee noted that for a considered contaminant, the occurrence data were not always submitted by Member States using a food classification system allowing a direct combination with the GEMS/Food consumption cluster diets.

The Committee recommends that Member States submit occurrence data in accordance with the Codex Alimentarius Commission classification for contaminants. The Committee also recommends that WHO update the GEMS/Food system for data reporting (OPAL) to simplify the submission of occurrence data in standard electronic files.

Acrylamide

The Committee recommends further efforts on developing and implementing mitigation methods for acrylamide in foods of major importance for dietary exposure.

To better estimate the risk from acrylamide in food for humans, the Committee recommends that longitudinal studies on intra-individual levels of acrylamide and glycidamide haemoglobin adducts be measured over time in relation to concurrent dietary exposure. Such data would provide a better estimate of acrylamide exposure for epidemiological studies designed to assess risk from the diet.

Arsenic

There is a need for validated methods for selective extraction and determination of inorganic arsenic in food matrices and for certified reference materials for inorganic arsenic.

There is a need for improved data on occurrence of different species of arsenic in, and their bioavailability from, different foods as consumed in order to

improve the estimates of dietary and systemic exposure. Further information on the toxicity of arsenic species found in food is also required.

The Committee recommends that future epidemiological studies of the health impacts of arsenic should incorporate appropriate measures of total exposure to inorganic arsenic, including from food and from water used in cooking and processing of food.

The Committee further recommends that epidemiological studies not only focus on relative risks, but also analyse and report the data such that they are suitable for estimating exposure levels associated with additional (lifetime) risks, so as to make their results usable for quantitative risk assessment.

DON

As DON-3-glucoside has been detected in cereals and beers and might therefore contribute to systemic exposure to DON, the Committee recommends that ADME studies be conducted on this substance.

Additional data on the occurrence of and the effects of processing on 3-Ac-DON, 15-Ac-DON and DON-3-glucoside are needed, as well as their co-occurrence with DON.

Mercury

There is a need for:

- validated analytical methods for both inorganic mercury and methylmercury applicable in several food matrices;
- more information on the inorganic mercury and methylmercury content of foods as consumed that mainly contribute to overall dietary exposure.

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Annex 1

Reports and other documents resulting from previous meetings of the Joint FAO/WHO Expert Committee on Food Additives

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Annex 2

Summary of toxicological evaluations

Acrylamide

Dietary exposure estimates:

Mean 0.001 mg/kg body weight (bw) per day

High 0.004 mg/kg bw per day

Effect	NOAEL/$BMDL_{10}$ (mg/kg bw per day)	MOE at		Conclusion/comments
		Mean dietary exposure	High dietary exposure	
Morphological changes in nerves in rats	0.2 (NOAEL)	200	50	The Committee noted that while adverse neurological effects are unlikely at the estimated average exposure, morphological changes in nerves cannot be excluded for individuals with a high dietary exposure to acrylamide.
Mammary tumours in rats	0.31 ($BMDL_{10}$)	310	78	The Committee considered that for a compound that is both genotoxic and carcinogenic, these MOEs indicate a health concern.
Harderian gland tumours in mice	0.18 ($BMDL_{10}$)	180	45	

$BMDL_{10}$, lower limit on the benchmark dose for a 10% response; bw, body weight; MOE, margin of exposure; NOAEL, no-observed-adverse-effect level.

Arsenic

The inorganic arsenic lower limit on the benchmark dose for a 0.5% increased incidence of lung cancer ($BMDL_{0.5}$) was determined from epidemiological studies to be 3.0 µg/kg bw per day (2–7 µg/kg bw per day based on the range of estimated total dietary exposure) using a range of assumptions to estimate total dietary exposure to inorganic arsenic from drinking-water and food. The Committee noted that the provisional tolerable weekly intake (PTWI) of 15 µg/kg bw (equivalent to 2.1 µg/kg bw per day) is in the region of the $BMDL_{0.5}$ and therefore was no longer appropriate. The Committee withdrew the previous PTWI.

Deoxynivalenol (DON)

As 3-acetyl-deoxynivalenol (3-Ac-DON) is converted to deoxynivalenol (DON) in vivo and therefore contributes to the total DON-induced toxicity, the Committee decided to convert the provisional maximum tolerable daily intake (PMTDI) for DON to a group PTMDI of 1 µg/kg bw for DON and its acetylated derivatives (3-Ac-DON and 15-Ac-DON). In this regard, the Committee considered the toxicity of the acetylated derivatives to be equal to that of DON. The Committee concluded that, at this time, there was insufficient information to include DON-3-glucoside in the group PMTDI.

The Committee derived a group acute reference dose (ARfD) of 8 µg/kg bw for DON and its acetylated derivatives using the lowest lower limit on the benchmark dose for a 10% response ($BMDL_{10}$) of 0.21 mg/kg bw per day for emesis in pigs. Limited data from human case reports indicated that dietary exposures to DON up to 50 µg/kg bw per day are not likely to induce emesis.

The Committee concluded that all of the mean estimates of national exposure to DON were below the group PMTDI of 1 µg/kg bw. National reports showed dietary exposures that were above 1 µg/kg bw per day in only a few cases, only for children at upper percentiles. For acute dietary exposure, the estimate of 9 µg/kg bw per day, based on high consumption of bread and a regulatory limit for DON of 1 mg/kg food, was close to the group ARfD.

Group PTMDI: 1 µg/kg bw for DON and its acetylated derivatives

Group ARfD: 8 µg/kg bw for DON and its acetylated derivatives

Furan

Dietary exposure estimates:

Mean 0.001 mg/kg bw per day

High 0.002 mg/kg bw per day

Effect	$BMDL_{10}$ (mg/kg bw per day)	MOE at		Conclusion/comments
		Mean dietary exposure	High dietary exposure	
Hepatocellular adenomas and carcinomas in female mice	1.3	1300	650	The Committee considered that these MOEs indicate a human health concern for a carcinogenic compound that might act via a DNA-reactive genotoxic metabolite.

$BMDL_{10}$, lower limit on the benchmark dose for a 10% response; bw, body weight; DNA, deoxyribonucleic acid; MOE, margin of exposure.

Mercury

The Committee established a PTWI for inorganic mercury of 4 μg/kg bw. The previous PTWI of 5 μg/kg bw for total mercury, established at the sixteenth meeting, was withdrawn.

The new PTWI for inorganic mercury was considered applicable to dietary exposure to total mercury from foods other than fish and shellfish. The upper limits of estimates of average dietary exposure to total mercury from foods other than fish and shellfish for adults (1 μg/kg bw per week) and for children (4 μg/kg bw per week) were at or below the PTWI.

PTWI: 4 μg/kg bw for inorganic mercury

Perchlorate

The Committee established a PMTDI of 0.01 mg/kg bw for perchlorate. The estimated dietary exposures of 0.7 μg/kg bw per day (highest) and 0.1 μg/kg bw per day (mean), including both food and drinking-water, are well below the PMTDI. The Committee considered that these estimated dietary exposures were not of health concern.

PMTDI: 0.01 mg/kg bw

Annex 3

Countries in the 13 GEMS/Food consumption cluster diets

Final cluster	Country	Final cluster	Country
A	Angola	C	Kuwait
A	Burundi	C	Libyan Arab Jamahiriya
A	Cameroon	C	Morocco
A	Central African Republic	C	Saudi Arabia
A	Comoros	C	Syrian Arab Republic
A	Côte d'Ivoire	C	Tunisia
A	Djibouti		
A	Eritrea	D	Albania
A	Ethiopia	D	Armenia
A	Gabon	D	Azerbaijan
A	Guinea	D	Belarus
A	Guinea-Bissau	D	Bosnia and Herzegovina
A	Liberia	D	Bulgaria
A	Madagascar	D	Georgia
A	Mauritius	D	Iran, Islamic Republic of
A	Rwanda	D	Kazakhstan
A	Sao Tome and Principe	D	Kyrgyzstan
A	Seychelles	D	Montenegro
A	Sierra Leone	D	Republic of Moldova
A	Somalia	D	Romania
A	Uganda	D	Russian Federation
A	Yemen	D	Serbia
		D	Tajikistan
B	Cyprus	D	The former Yugoslav Republic of Macedonia
B	Greece	D	Turkmenistan
B	Israel	D	Ukraine
B	Italy	D	Uzbekistan
B	Lebanon		
B	Portugal	E	Austria
B	Spain	E	Belgium
B	Turkey	E	Croatia
B	United Arab Emirates	E	Czech Republic
		E	Denmark
C	Algeria	E	France
C	Egypt	E	Germany
C	Iraq	E	Hungary
C	Jordan	E	Ireland

Final cluster	Country	Final cluster	Country
E	Luxembourg	I	Cape Verde
E	Malta	I	Ghana
E	Netherlands	I	Kenya
E	Poland	I	Lesotho
E	Slovakia	I	Malawi
E	Slovenia	I	Mozambique
E	Switzerland	I	Namibia
E	United Kingdom of Great Britain and Northern Ireland	I	South Africa
		I	Swaziland
F	Estonia	I	Togo
F	Finland	I	United Republic of Tanzania
F	Iceland	I	Zambia
F	Latvia	I	Zimbabwe
F	Lithuania		
F	Norway	J	Burkina Faso
F	Sweden	J	Chad
		J	Congo
G	Afghanistan	J	Democratic Republic of the Congo
G	Bangladesh	J	Gambia
G	Cambodia	J	Mali
G	China	J	Mauritania
G	India	J	Niger
G	Indonesia	J	Nigeria
G	Lao People's Democratic Republic	J	Senegal
G	Malaysia	J	Sudan
G	Mongolia		
G	Myanmar	K	Antigua and Barbuda
G	Nepal	K	Bahamas
G	Pakistan	K	Barbados
G	Sri Lanka	K	Belize
G	Thailand	K	Bermuda
G	Viet Nam	K	Brazil
		K	Colombia
H	Bolivia, Plurinational State of	K	Costa Rica
H	El Salvador	K	Cuba
H	Guatemala	K	Dominica
H	Haiti	K	Dominican Republic
H	Honduras	K	Ecuador
H	Mexico	K	Grenada
H	Nicaragua	K	Guyana
H	Panama	K	Jamaica
H	Peru	K	Netherlands Antilles
H	Saint Kitts and Nevis	K	Saint Lucia
H	Saint Vincent and the Grenadines	K	Suriname
		K	Trinidad and Tobago
I	Benin	K	Venezuela (Bolivarian Republic of)
I	Botswana		

Final cluster	Country	Final cluster	Country
L	Brunei Darussalam	L	Solomon Islands
L	Democratic People's Republic of Korea	L	Vanuatu
L	Fiji		
L	French Polynesia	M	Argentina
L	Japan	M	Australia
L	Kiribati	M	Canada
L	Maldives	M	Chile
L	New Caledonia	M	New Zealand
L	Papua New Guinea	M	United States of America
L	Philippines	M	Uruguay
L	Republic of Korea		

Note: The consumption figures of the 13 GEMS/Food consumption cluster diets represent mainly raw commodities, with some processed and semiprocessed foods. They are found at http://www.who.int/entity/foodsafety/chem/ClusterDietsAug06.xls.

Source: Adapted from http://www.who.int/foodsafety/chem/countries.pdf

This report represents the conclusions of a Joint FAO/WHO Expert Committee convened to evaluate the safety of various food contaminants, with the aim to advise on risk management options for the purpose of public health protection.

The first part of the report contains a general discussion of the principles governing the toxicological evaluation of contaminants and assessments of dietary exposure. A summary follows of the Committee's evaluations of technical, toxicological and dietary exposure data for certain food contaminants: acrylamide, arsenic, deoxynivalenol, furan, mercury and perchlorate.

Annexed to the report are tables summarizing the Committee's recommendations for dietary exposures and toxicological evaluations of the food contaminants considered.

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