WHO/PCS/06.1

Pesticide residues in food - 2004

Joint FAO/WHO Meeting on Pesticide Residues

EVALUATIONS 2004

Part II—Toxicological

IPCS

International Programme on Chemical Safety

WORLD HEALTH ORGANIZATION

Pesticide residues in food—2004

Toxicological evaluations

Sponsored jointly by FAO and WHO
With the support of the International Programme on Chemical Safety (IPCS)

Joint Meeting of the
FAO Panel of Experts on Pesticide Residues in Food and the Environment
and the
WHO Core Assessment Group

Rome, Italy, 20–29 September 2004

WHO/PCS/06.1

WORLD HEALTH ORGANIZATION

WHO Library Cataloguing-in-Publication Data

Joint Meeting of the FAO Panel of Experts on Pesticides Residues in Food and the Environment and the WHO Core Assessment Group (2004 : Rome, Italy) Pesticide residues in food : 2004 : toxicological evaluations : part II / Joint Meeting of the FAO Panel of Experts on Pesticides Residues in Food and the Environment and the WHO Core Assessment Group, Rome, Italy 20–29 September 2004.

1.Pesticide residues—toxicity. 2.Food contamination. 3.No-observed-adverse-effect level. I.FAO Panel of Experts on Pesticide Residues in Food and the Environment. II.WHO Core Assessment Group on Pesticide Residues. III.Title. IV.Pesticide residues in food : evaluations : 2004 : part II—toxicological.

ISBN 92 4 166520 3 (NLM classification: WA 240)
ISBN 978 92 4 166520 9

This report contains the collective views of two international groups of experts and does not necessarily represent the decisions nor the stated policy of the Food and Agriculture Organization of the United Nations or the World Health Organization.

The preparatory work for the toxicological evaluations of pesticide residues carried out by the WHO Expert Group on Pesticide Residues for consideration by the FAO/WHO Joint Meeting on Pesticide Residues in Food and the Environment is actively supported by the International Programme on Chemical Safety within the framework of the Inter-Organization Programme for the Sound Management of Chemicals.
The **International Programme on Chemical Safety** (IPCS), established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organization (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessing the risk to human health and the environment to exposure from chemicals, through international peer-review processes as a prerequisite for the promotion of chemical safety, and to provide technical assistance in strengthening national capacities for the sound management of chemicals.
The Inter-Organization Programme for the Sound Management of Chemicals (IOMC) was established in 1995 by UNEP, the Food and Agriculture Organization of the United Nations, WHO, the United Nations Industrial Development Organization, and the Organisation for Economic Co-operation and Development (Participating Organizations), following recommendations made by the 1992 United Nations Conference on the Environment and Development to strengthen cooperation and increase coordination in the field of chemical safety. The purpose of the IOMC is to promote coordination of the policies and activities pursued by the Participating Organizations, jointly or separately, to achieve the sound management of chemicals in relation to human health and the environment.

Typeset in Hong Kong
Printed in Malta

TABLE OF CONTENTS

* First full evaluation
** Evaluated within the periodic review programme of the Codex Committee on Pesticide Residues

2004 Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group

Rome, 20–29 September 2004

PARTICIPANTS

Toxicological Core Assessment Group

Professor Alan R. Boobis, Experimental Medicine & Toxicology, Division of Medicine, Faculty of Medicine, Imperial College London, London, England (*WHO Chairman*)

Dr Les Davies, Science Strategy and Policy, Office of Chemical Safety, Australian Government Department of Health and Ageing, Woden, ACT, Australia

Dr Vicki L. Dellarco, United States Environmental Protection Agency, Office of Pesticide Programs, Health Effects Division, Washington, DC, USA (*WHO Rapporteur*)

Dr Helen Hakansson, Institute of Environmental Medicine, Karolinska Institutet, Unit of Environmental Health Risk Assessment, Stockholm, Sweden

Dr Angelo Moretto, Dipartimento Medicina Ambientale e Sanità Pubblica, Università di Padova, Padova, Italy

Dr Roland Solecki, Pesticides and Biocides Division, Federal Institute for Risk Assessment, Berlin, Germany

Dr Maria Tasheva, Laboratory of Toxicology, National Center of Hygiene, Medical Ecology and Nutrition, Sofia, Bulgaria

FAO Panel of Experts on Pesticide Residues in Food and the Environment

Dr Ursula Banasiak, Federal Institute for Risk Assessment, Berlin, Germany

Dr Eloisa Dutra Caldas, University of Brasilia, College of Health Sciences, Pharmaceutical Sciences Department, Brasília/DF, Brazil

Dr Stephen Funk, Health Effects Division, United States Environmental Protection Agency, Washington, DC, USA (*Chairman*)

Mr Denis J. Hamilton, Biosecurity, Department of Primary Industries and Fisheries, Brisbane, Australia (*Rapporteur*)

Dr Bernadette C. Ossendorp, Centre for Substances and Integrated Risk Assessment (SIR), National Institute of Public Health and the Environment (RIVM), Bilthoven, Netherlands

Dr Yukiko Yamada, Research Planning and Coordination Division, National Food Research Institute, Tsukuba, Japan

Secretariat

Dr Arpàd Ambrus, Central Service for Plant Protection and Soil Conservation, Plant and Soil Protection Directorate, Budapest, Hungary (*FAO Consultant*)

Dr Andrew Bartholomaeus, Therapeutic Goods Administration, Commonwealth Department of Health and Ageing, Woden, ACT, Australia (*WHO Temporary Adviser*)

Dr Lourdes Costarrica, Food and Nutrition Division, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Staff Member*)

Mr Bernard Declercq, Epinay sur Orge, France (*FAO Consultant*)

Dr Ghazi Dannan, Office of Pesticide Programs, United States Environmental Protection Agency, Washington, DC, USA (*WHO Temporary Adviser*)

Dr Ian C. Dewhurst, Pesticides Safety Directorate, Mallard House, King's Pool, 3 Peasholme Green, York YO1 7PX, England (*WHO Temporary Adviser*)

Dr Salwa Dogheim, Central Laboratory of Residue Analysis of Pesticides and Heavy Metals in Food, Agriculture Research Center, Ministry of Agriculture, Heliopolis, Cairo, Egypt (*FAO Consultant*)

Professor P.K. Gupta, Toxicology Consulting Services Inc., Bareilly, UP, India (*WHO Temporary Adviser*)

Dr Yibing He, Pesticide Residue Division, Institute for the Control of Agrochemicals, Ministry of Agriculture, Cheoyang District, Beijing, China (*FAO Consultant*)

Dr H. Jeuring, Chairman, Codex Committee on Pesticide Residues, Senior Public Health Officer, Food and Consumer Product Safety Authority, The Hague, Netherlands (*WHO Temporary Adviser*)

Mr David Lunn, Programme Manager (Residues-Plants), Dairy and Plant Products Group, New Zealand Food Safety Authority, Wellington, New Zealand (*FAO Consultant*)

Mr Antony F. Machin, London, England (*FAO Editor*)

Dr Dugald MacLachlan, Australian Quarantine and Inspection Service, Australian Department of Agriculture, Fisheries and Forestry, Kingston, ACT, Australia (*FAO Consultant*)

Dr Timothy C. Marrs, Food Standards Agency, Aviation House, London, England (*WHO Temporary Adviser*)

Dr Jeronimas Maskeliunas, Joint FAO/WHO Food Standards Programme, Food and Nutrition Division, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Staff Member*)

Dr Heidi Mattock, Illkirch-Graffenstaden, France (*WHO Editor*)

Dr Douglas B. McGregor, Aberdour, Scotland (*WHO Temporary Adviser*)

Dr Rudolf Pfeil, Pesticides and Biocides Division, Federal Institute for Risk Assessment, Berlin (*WHO Temporary Adviser*)

Mr Tsuyoshi Sakamoto, Agricultural Chemicals Inspection Station, Kodaira, Tokyo, Japan (*FAO Consultant*)

Dr Atsuya Takagi, Division of Toxicology, Biological Safety Research Centre, National Institute of Health Sciences, Setagaya-ku, Tokyo, Japan (*WHO Temporary Adviser*)

Dr Amelia Tejada, Pesticide Management Group, Plant Protection Service, Plant Production and Protection Division, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Joint Secretary*)

Dr Angelika Tritscher, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland (*WHO Joint Secretary*)

Dr Gero Vaagt, Pesticide Management Group, Food and Agriculture Organization of the United Nations, Rome, Italy (*FAO Staff Member*)

Dr Gerrit Wolterink, Centre for Substances & Risk Assessment, National Institute for Public Health and the Environment (RIVM), Bilthoven, Netherlands (*WHO Temporary Adviser*)

Dr Jürg Zarn, Swiss Federal Office of Public Health, Food Toxicology Section, Zurich, Switzerland (*WHO Temporary Adviser*)

Abbreviations used

ADI	acceptable daily intake
ARfD	acute reference dose
AUC	area under the curve of concentration–time
bw	body weight
DMSO	dimethylsulfoxide
ECG	electrocardiogram
F_0	parental generation
F_1	first filial generation
F_2	second filial generation
FIFRA	Federal Insecticide, Fungicide, and Rodenticide Act
FOB	functional observational battery
GLP	good laboratory practice
HDW	haemoglobin concentration distribution width
HPLC	high-performance liquid chromatography
IPCS	International Programme on Chemical Safety
ISO	International Organization for Standardization
IUPAC	International Union of Pure and Applied Chemistry
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LOAEL	lowest-observed-adverse-effect level
MS	mass spectrometry
MCV	mean cell volume
MCH	mean corpuscular haemoglobin
MCHC	mean corpuscular haemoglobin concentration
MRL	maximum residue level
NOAEC	no-observed-adverse-effect concentration
NOAEL	no-observed-adverse-effect level
NMR	nuclear magnetic resonance
OECD	Organisation for Economic Co-operation and Development
ppm	parts per million
TLC	thin-layer chromatography
TMDI	theoretical maximum daily intake
QA	quality assurance
WHO	World Health Organization
w/v	weight for volume
w/w	weight for weight

Introduction

The toxicological monographs and monograph addenda contained in this volume were prepared by a WHO Core Assessment Group that met with the FAO Panel of Experts on Pesticide Residues in Food and the Environment in a Joint Meeting on Pesticide Residues (JMPR) in Rome, Italy, on 20–29 September 2004.

Two of the compounds evaluated by the Core Assessment Group at the Meeting, fludioxinil and trifloxystrobin, were evaluated for the first time. The other 11 substances had been evaluated at previous meetings. For six of these, only information received since the previous evaluations is summarized in "monograph addenda". Of these, bentazone, captan, dimethipin, folpet, fenpropimorph and fenpyroximate were evaluated for establishment of an acute reference dose. The appropriate earlier documents on the six compounds should be consulted in order to obtain full toxicological profiles. Toxicological monographs were prepared on glyphosate, phorate, pirimicarb, propiconazole, triademefon/triademenol, summarizing new data and, where relevant, incorporating information from previous monographs and addenda. Reports and other documents resulting from previous Joint Meetings on Pesticide Residues are listed in Annex 1.

The report of the Joint Meeting has been published by the FAO as *FAO Plant Production and Protection Paper* 178. That report contains comments on the compounds considered, acceptable daily intakes established by the WHO Core Assessment Group, and maximum residue limits established by the FAO Panel of Experts. Monographs on residues prepared by the FAO Panel of Experts are published as a companion volume, as *Evaluations 2004, Part I, Residues*, in the FAO Plant Production and Protection Paper series.

The toxicological monographs and addenda contained in this volume are based on working papers that were prepared by temporary advisers before the 2004 Joint Meeting. A special acknowledgement is made to those advisers.

The preparation and editing of this volume was made possible by the technical and financial contributions of the lead institutions of the International Programme on Chemical Safety (IPCS), which supports the activities of the JMPR. The designations employed and the presentation of the material in this publication do not imply the expression of any opinion whatsoever on the part of the Central Unit of the IPCS concerning the legal status of any country, territory, city or area or of its authorities, nor concerning the delimitation of its frontiers or boundaries. The mention of specific companies or of certain manufacturers' products does not imply that they are endorsed or recommended by the IPCS in preference to others of a similar nature that are not mentioned.

Any comments or new information on the biological properties or toxicity of the compounds included in this volume should be addressed to: Joint WHO Secretary of the Joint FAO/WHO Meeting on Pesticide Residues, International Programme on Chemical Safety, World Health Organization, 20 Avenue Appia, 1211 Geneva, Switzerland.

TOXICOLOGICAL MONOGRAPHS AND MONOGRAPH ADDENDA

BENTAZONE (addendum)

First draft prepared by
T.C. Marrs
Food Standards Agency, London, England

Explanation

Bentazone was first evaluated by the Joint Meeting in 1991 (Annex 1, reference 62), when an acceptable daily intake (ADI) of 0–0.1 mg/kg bw was established. This was on the basis of the no-observed-adverse-effect level (NOAEL) of 9 mg/kg bw per day in a long-term study in rats and a safety factor of 100. Further data were made available to the Meeting in 1998, including observations in humans and a 90-day study in rats fed with 6-hydroxybentazone, the metabolite of bentazone. Data on the genotoxicity of 6-hydroxybentazone were also supplied. The Meeting concluded that 6-hydroxybentazone was less toxic than bentazone and the ADI of 0–0.1 mg/kg bw was maintained. Data were not evaluated to establish an acute reference dose (ARfD). The present Meeting re-evaluated some of the previously evaluated data to establish an ARfD, and two cases of acute human poisoning were described.

Evaluation for acute reference dose

1. Toxicological studies

1.1 Short-term studies of toxicity

Mice

In a briefly-reported 30-day study of toxicity, groups of six male and six female B6C3F$_1$/CRJ mice were given diets containing bentazone (purity, 93.9%) at a concentration of 400, 2000, 5000 or 10 000 ppm, equal to 90, 407, 905 and 1469 mg/kg bw per day for males and 100, 487, 1004 and 1663 mg/kg bw per day for females. The mice were observed daily and weighed twice per week. Food and water consumption were measured twice per week. After 30 days, the animals were killed, subjected to necropsy and selected organs were weighed and processed for histopathology. Blood was taken from some mice (three out of six controls of each sex, three out of six male mice at 2000 ppm and two out of six female mice at 2000 and 5000 ppm) for measurement of clinical pathology parameters. At 10 000 ppm, all mice died within 30 days, while at 5000 ppm, six males and four females died. At 5000 and 10 000 ppm, the mice showed depression, skin pallor and low skin

temperature. At 5000 and 10 000 ppm, decreases in body-weight gain, food consumption, and water consumption were seen in both sexes. At autopsy of the decedents, which were all mice that had been fed bentazone at 5000 or 10 000 ppm, haemorrhages were seen in subcutaneous tissue, the pia, the lungs, the thoracic, pericardial and abdominal cavities, the thymus, the orbits, and skeletal muscles. Prothrombin time and partial thromboplastin time were prolonged at 2000 ppm in both sexes and at 5000 ppm in females. On histopathological examination of animals receiving bentazone at 5000 ppm and 10 000 ppm, there was haemosiderosis and extramedullary haematopoiesis in the spleen, haemorrhage and haemosiderosis in cardiac muscle, and haemorrhages in the cerebral cortex and pia. The NOAEL for the study was 400 ppm (equal to 90 and 100 mg/kg bw per day in males and females respectively) on the basis of prolonged prothrombin and partial thromboplastin times at 2000 ppm (Anonymous, 1981).

Rats

In a 13-week study of oral toxicity, groups of 10 male and 10 female Wistar KFM-Han rats were given diets containing bentazone (purity, 97.8%) at a concentration of 0, 400, 1200 or 3600 ppm (Tennekes et al., 1987). These dietary concentrations produced intakes of 0, 25.3, 77.8 and 243 mg/kg bw per day for males and 0, 28.9, 86.1 and 258 mg/kg bw per day for females. For the controls and at the highest dietary concentration, additional groups of 10 rats of each sex received the diets for 13 weeks and were then maintained for 4 weeks, to study the reversibility of any effects observed ("recovery" groups). The rats were observed twice daily, and body weights were recorded before the start of the study and then weekly thereafter, except in the recovery groups, for which body weights were not recorded in week 15. Food consumption was recorded weekly and ophthalmoscopic examination was carried out before the start and at the termination of the study for all groups, including those maintained for 4 weeks. Likewise, samples of blood and urine were taken at the end of the study from animals in all groups. The blood was used for haematological investigations and for measurements of clinical chemistry parameters. At necropsy, the weights of the adrenals, livers, kidneys and testes were determined. Sections of the lung, liver, kidney and testis from animals in all groups were processed for histopathological examination, as were any macroscopically abnormal organs. Sections of other selected organs from animals in the control group and at highest dietary concentration were also prepared and processed for histopathological examination. The same organs were retained and fixed from animals fed diets containing bentazone at the intermediate concentrations with the intention that they should only be processed and examined if toxicity at the highest dietary concentration was seen in the target organs. There were three deaths during the study, all among rats receiving bentazone at the highest dietary concentration. One was a male, and two were females, one of which died during anaesthesia on the day of necropsy. No clinical signs of toxicity were seen. Food consumption was similar in all groups. Body-weight gain was similar in all groups of males. Body-weight gain was lower in females at the highest dietary concentration than in the controls; in the recovery group, during the 4-week recovery period, body-weight gain was similar to that of the controls, but body weight was still below that of the controls at termination. Body-weight gain in animals in the groups receiving diets containing bentazone at the lowest or intermediate concentration was similar to that in the controls. No abnormalities were seen on ophthalmoscopic examination. At the highest dietary concentration in males, prolonged thromboplastin and partial thromboplastin times were observed. This proved to be reversible in the animals that experienced a 4-week recovery period. An increase in the concentration of albumin and in the albumin : globulin ratio at dietary concentrations of 1200 and 3600 ppm was found in males after 13

weeks; this was not found at 17 weeks, i.e. after the recovery period. Changes in the concentration of albumin and in the albumin : globulin ratio were not seen in females. An increase in cholesterol was observed in females receiving bentazone at a dietary concentration of 3600 ppm. An increase in urine output and a decrease in specific gravity were seen in both sexes at the highest dietary concentration. An increase in absolute and relative kidney weight was found in males at the highest dietary concentration, while an increase in kidney weight, relative but not absolute, was found in females at highest dietary concentration. No treatment-related macroscopic or microscopic abnormality was seen at necropsy. The NOAEL was 400 ppm (equal to 25.3 and 28.9 mg/kg bw per day for males and females respectively) on the basis of clinical chemistry changes at the next highest dietary concentration (Tennekes et al., 1987).

Dogs

In a 13-week study of toxicity, groups of three male and three female beagles were given diets containing bentazone (technical grade; purity unstated) at a concentration of 0, 100, 300, 1000 or 3000 ppm (corresponding to intakes of 4.0, 12.0, 39.6 and 114 mg/kg bw per day). The animals were observed daily. Body weight was measured once per week, water and food consumption were measured daily. Blood was taken for measurement of haematological and clinical chemistry parameters before the start of the study and at weeks 6 and 13. Urine analysis was also undertaken. In addition to the usual clinical chemistry studies, a bromsulfthalein retention test was carried out before the start of treatment and at weeks 6 and 13. Electrocardiography was also carried out before the start of treatment and at weeks 6 and 13. Immediately before termination, hearing was tested, and ophthalmoscopic examination was undertaken. After termination, selected organs were weighed and processed for histopathological examination. Three animals at the highest dietary concentration died in a coma (one male, two females) during or after week 11. Significant clinical effects were not seen early in the study, although vomiting was later observed at 3000 ppm in all three males. Sedation, attacks of hyperactivity, ataxia, prostration and tremor were also observed at 3000 ppm. Diarrhoea, sometimes bloody, was observed during the second half of the study. One male at 1000 ppm showed sedation and this dog developed an ulcer later in the study. At 3000 ppm, but not at lower doses, body-weight loss and reduced food consumption were reported. Bilateral haemorrhagic conjunctivitis was observed in all animals at 3000 ppm. Concentration of haemoglobin, erythrocyte count, and erythrocyte volume fraction were decreased and the prothrombin time and bleeding time were prolonged at this, but not at lower dietary concentrations. The reticulocyte count was elevated and the platelet count was reduced at the highest dietary concentration. The erythrocyte sedimentation rate was increased. Clinical chemistry changes were seen only at the highest dietary concentration; there was an increase in activity of the alanine and aspartate aminotransferases and alkaline phosphatase. The blood concentration of urea nitrogen was increased, as was that of total bilirubin, while the concentration of total protein was decreased, electrophoresis showing a decrease in the albumin fraction, an increase in α_1 globulin and a decrease in gamma globulin. The result of the bromsulfthalein test (% at 15 min) was raised.

At the lower doses, necropsy showed the animals to be macroscopically normal, while at the highest dose, cachexia was seen, accompanied by conjunctivitis and stomatitis and all animals had pale livers and most had pale kidneys. At the highest dose, substantially increased relative weights were recorded for livers, kidneys and adrenals, and marginally increased relative weights for spleens, lungs, thymuses, thyroids and brains (the relative weights for the survivors of both sexes were added together and compared with those for

the controls for the two sexes). No test material-related changes were found in any organ at histopathological examination at the lower dietary concentrations. At the highest dose, however, there was congestion and necrosis in the liver, together with fatty change, evidence of extramedullary haematopoiesis in the spleen, fatty change in the myocardium and cloudy swelling of the renal tubular cells. The NOAEL was 300 ppm, equal to 12.0 mg/kg bw per day, on the basis of sedation and ulceration and alopecia in the leg of one dog at 1000 ppm (Leuschner et al., 1970).

In a 1-year study in beagle dogs, four groups of six males and six females were given diets containing bentazone (purity, 97.8%) at a concentration of 0, 100, 400 or 1600 ppm (equal to 0, 3.2, 13.1 and 52.3 mg/kg bw per day) for 52 weeks. Animals were observed twice per day for any mortality and once per day for clinical signs. Food consumption was recorded daily and body weight was recorded weekly. Hearing tests were performed before the start of the study and after 52 weeks of treatment. Ophthalmoscopy was carried out before the start of the study, and at 13, 26 and 52 weeks after the start of treatment. The faeces of all the beagles in the control group and at the highest dietary concentration were examined for occult blood at week 14 of treatment. Samples of blood and urine were taken before the start of the study and at 13, 26 and 52 weeks after the start of treatment. Additional blood samples were taken from one dog (a male at the highest dietary concentration) at 7 and 8 weeks after the start of treatment. The blood samples were used for measurement of haematological and clinical chemistry parameters. The animals were killed after 52 weeks of treatment and selected organs were weighed. Selected organs were taken and processed for histopathological examination. No animals died during the study. Clinical signs (appearance of emaciation and dehydration in one male dog and hyperaemia and alopecia in another) were seen at the highest dietary concentration. Diarrhoea, on some occasions bloody, was seen in another male and in a female; in the latter case this was associated with a marked reduction in food consumption, gastroenteritis was diagnosed and the dog was treated with an antibiotic. Weight loss early during the exposure period was seen in two males and two females, all receiving bentazone at 1600 ppm; in all except one case, recovery occurred. Hearing tests and ophthalmoscopy did not reveal any clinical effects of the treatment. Faecal occult blood was not seen in the controls or dogs at the highest dose at week 14. No intergroup differences in mean haematological variables were seen. However, anaemia was seen in the male with diarrhoea (there was recovery after a short treatment-free period on days 44–49 inclusive). Anaemia was also seen in one female receiving bentazone at 1600 ppm. No findings of toxicological significance were seen on the results of clinical chemistry or urine analysis. There was no evidence of any test material-related effect on organ weights, and no histopathological change that could be ascribed to bentazone. The NOAEL for the study was 400 ppm, equal to 13.1 mg/kg bw per day, on the basis of clinical signs, weight loss and anaemia at the highest dietary concentration (Allen et al., 1989).

1.2 Developmental toxicity

Rats

In a study of developmental toxicity, groups of 26–29 impregnated Sprague-Dawley rats were given bentazone (purity, 92.5%) at a dose of 22.2, 66.7 or 200 mg/kg bw per day orally by gavage from day 6 to day 15 post coitum. Control groups received vehicle only or were untreated. The animals were observed daily. Body weight was determined three times per week, on day zero of pregnancy and also on days 6, 11, 15 and 20 post coitum. On day 20 post coitum, the rats were killed and the fetuses were delivered by caesarian section. Dams were examined macroscopically and the uteri were removed. The conception

rate and the number of corpora lutea and implantations were determined. The number of viable fetuses and their sex, weight and length were established. Dead implantations (early resorptions, intermediate resorptions, late resorptions and dead fetuses) were counted. The placentas were weighed. Two thirds of the fetuses were used for skeletal examination after staining with alizarin red. The remainder were processed for brain and visceral examination. Mortality was not observed. No clinical effects were seen in the dams, and the test material did not affect maternal body-weight gain. At autopsy, no macroscopic abnormality of the dams was observed. The conception rates, and numbers of dead implantations and viable fetuses were not affected by the treatment. Body weight and length of the fetuses was unaffected and there were no intergroup differences in numbers of anomalies observed. Placental weight was decreased in comparison to that of the untreated controls at the two higher doses: as no difference was observed in comparison with the vehicle controls, this finding is of dubious clinical significance. The NOAEL for both maternal and fetal toxicity was 200 mg/kg bw per day, the highest dose tested (Hofmann & Merkle, 1978a).

In a study of developmental toxicity, groups of 23 impregnated CD(SD) rats were given diets containing bentazone (purity, 93.9%) at a concentration of 0, 2000, 4000 or 8000 ppm (equal to 0, 162, 324 and 631 mg/kg bw per day) throughout pregnancy (days 0–21 of gestation). The rats were observed twice per day throughout the study. Body weights and food consumption were measured daily. On day 21 of gestation, the rats were killed and the main organs were examined grossly. The numbers of implantations, corpora lutea, and fetuses were counted and the placentas and placental membranes were observed. Embryonic and fetal mortalities were recorded. The uteri were weighed. Viable fetuses were weighed, sexed and external abnormalities were sought. Half the fetuses were fixed and stained with alizarin red for skeletal examination, while the other half were used for examination of the brain and viscera. Maternal mortality was not observed. No abnormal clinical signs were seen at 2000 and 4000 ppm, while, haematuria, nasal haemorrhage, skin pallor and piloerection were seen at 8000 ppm during late pregnancy. Weight gain and food consumption were both decreased at 8000 ppm. Increased water consumption was seen at 4000 and 8000 ppm. Emaciation and intrauterine haemorrhage were seen at 8000 ppm in one rat. Numbers of corpora lutea, implantation rates, and embryo-fetal mortality were similar in all groups. The numbers of viable fetuses were similar in all groups. The weight of amniotic fluid was increased at 4000 and 8000 ppm. At 8000 ppm, fetal weights were decreased and petechiae were found in the liver. The number of ossified cervical vertebrae was significantly lower in the group receiving bentazone at 8000 ppm. The NOAEL for maternal toxicity was 4000 ppm (equal to 324 mg/kg bw per day) on the basis of decreases in water consumption, food consumption and body weight at 8000 ppm. The NOAEL for fetal toxicity was 4000 ppm (equal to 324 mg/kg bw per day) on the basis of decreased fetal weights, decreased ossification of cervical vertebrae and fetal liver petechiae at 8000 ppm. Bentazone was not teratogenic (Itabashi et al., 1982).

In a study of developmental toxicity, groups of 25 female impregnated Wistar/HAN rats were given bentazone (purity, 97.8%) at a dose of 0, 40, 100 or 250 mg/kg bw per day by gavage from day 6 to day 15 post coitum; an additional group received vehicle only. During the study, the rats were observed twice per day. Body weights were recorded daily and food consumption was recorded on days 6, 11, 16, and 21 post coitum. At day 21 post coitum, the rats were killed, the fetuses were removed by caesarian section and the uteri were weighed. Half the fetuses from each litter were fixed and processed for examination of viscera and brains. The other half were fixed and stained with alizarin red for examination of the skeletons. No deaths were recorded. No abnormal clinical signs were seen in the

dams. No intergroup differences in the body-weight gain of the dams were seen except at 100 mg/kg bw per day, and this was attributed to a higher number of offspring per dam in this group. There was a small reduction in food consumption at the highest dose between days 6 and 11 post coitum. There was an increase in postimplantation loss at the highest dose (22% of implantation versus 7.4% in the controls) and a correspondingly reduced number of live fetuses. Externally the fetuses appeared to be normal. The sex ratio of fetuses was similar in all groups, but the mean body weight of the fetuses in the group receiving the highest dose was reduced. No abnormal findings were seen on visceral examination, but incompletely ossified fetal skeletons were seen at the highest dose on skeletal examination. The NOAEL was 100 mg/kg bw per day for maternal toxicity (decreased food consumption) and fetal toxicity (postimplantation loss, reduced fetal weight and incompletely ossified fetal skeletons). Bentazone was not teratogenic (Becker et al., 1986).

Rabbits

In a study of developmental toxicity, groups of 15 Himalayan rabbits (ChBB : HM) were given bentazone (purity, 92.5%) at a dose of 50, 100 or 150 mg/kg bw per day by gavage on day 6 to day 18 post coitum. Two additional groups served as untreated controls or vehicle controls. The animals were observed daily for clinical signs and for mortality. Body weights and body-weight gains were measured each day and food consumption was estimated daily. At necropsy, the uterus was removed and the animals were examined for gross pathology. The number of corpora lutea, conception rate, the number of implantations (live and dead implantation and early, intermediate and late resorptions) and dead fetuses were determined. The fetuses were removed from the uterus by caesarian section and examined. The weight and length of the fetuses were measured and the placentas were weighed. The heads of the fetuses were fixed and transverse sections were made and examined, while skeletal assessment was undertaken by radiological examination. One dam in the untreated control group aborted. One death was seen in each of the groups receiving bentazone at 100 and 150 mg/kg bw per day (these dams had severe vaginal haemorrhages) and a dam in the group at 100 mg/kg bw per day bore six fetuses prematurely on day 26 after conception. A dam at 100 mg/kg bw per day aborted on day 26 or 27 post coitum. No other adverse clinical signs were noted and there was no test material-related effect on maternal body-weight gain. Food consumption was lower in the dosed groups and in the vehicle control groups than in the group of untreated controls. No test material-related macroscopical abnormalities were seen in the animals that were killed at study termination. No intergroup differences were seen in conception rate and numbers of implantations and corpora lutea. Fetal body weights were increased at 100 and 150 mg/kg bw per day; however, this is not likely to be an adverse effect. Fetal length and placental weight were not affected by treatment. There were no differences between the groups in the frequency of anomalies, variations and retardations obeserved. Accordingly, the NOAEL for maternal and fetal toxicity for bentazone was 150 mg/kg bw per day, the highest dose tested. Bentazone was not teratogenic (Hofmann & Merkle, 1978b).

In a study of developmental toxicity, groups of 16 female impregnated chinchilla (Kfm: CHIN) rabbits were given bentazone at a dose of 0, 75, 150 or 375 mg/kg bw per day (purity, 97.8%) by gavage from days 6 to 18 of gestation. The rabbits were observed twice per day and body weights were recorded daily. Food consumption was recorded on days 6, 11, 15, 19, 24 and 28 post coitum. On day 28 post coitum, the rabbits were killed and the fetuses removed by caesarian section. The internal organs of the dams were examined grossly and the uteri weighed. The fetuses were weighed, examined grossly and sexed. The

crania were examined for ossification and organs were examined. The trunks were processed and stained with alizarin red for skeletal examination. At the highest dose, abortion occurred in one dam, and total postimplantation loss was confirmed in this dam at necropsy. This was considered to be a test material-related effect in view of the results of a range-finding study (reference not available), in which postimplantation losses of 70% and 11.1% occurred at 450 mg/kg bw per day and 300 mg/kg bw per day, respectively. In the other groups, all the rabbits were pregnant. No clinical signs attributable to the test material were seen. Differences in body-weight gain between the groups were not seen. There was a reduction in food consumption at 375 mg/kg bw per day. No abnormality was seen at necropsy of the dams. Numbers of corpora lutea, implantations and pre-implantation loss were similar in all groups, as were numbers of dead fetuses and numbers of embryonic and fetal resorptions. Postimplantation loss was increased at 375 mg/kg bw per day; this was attributed to the total postimplantation loss occurring in one dam, which was thought to be test-material-related. The sex ratio of the fetuses did not differ between groups and no abnormal finding was seen on external, visceral or skeletal examination of the fetuses.

The NOAEL for both fetal and maternal toxicity was 150 mg/kg bw per day on the basis of reduction in maternal food consumption and increased postimplantation loss at 375 mg/kg bw per day. Bentazone was not teratogenic (Becker et al. 1987).

2. Observations in humans

Case reports of poisoning

A woman aged 59 years intentionally ingested 100–200 ml of a commercial preparation of bentazone. She had vomiting and diarrhoea, and was drowsy with muddled speech. After 2 days, she was admitted to hospital where she had a cardiac arrest and died. The precise cause of death could not be ascertained at autopsy, but bentazone, alcohol and desmethyl-citalopram were found in the blood (Müller et al., 2003).

Turcant et al. (2003) reported a fatal suicidal attempt using bentazone. A male farmer ingested 500 ml of a commercial preparation of bentazone. He had diarrhoea and vomiting and polypnoea. During transport to hospital he had breathing difficulty of sudden onset. Rigidity was seen. This was followed by heart failure and the patient died. Plasma and urine concentrations of bentazone were 1500 mg/l and 1000 mg/l, respectively. Various metabolites were also seen in the urine.

Comments

The oral median lethal dose (LD_{50}) for bentazone was 1200–2500 mg/kg bw in rats. In 13-week studies in mice, rats and dogs, interference with blood clotting was a consistently observed effect. There was prolongation of the prothrombin and partial thromboplastin times in mice and rats, and prolongation of the prothrombin and bleeding time in dogs. Additionally, extramedullary haematopoiesis, haemorrhage and haemosiderosis were found in mice at autopsy. Toxicological effects in rats were less dramatic, the NOAEL being identified on the basis of clinical chemistry changes. In dogs, clinical effects, such as hyperactivity, ataxia, prostration and tremor, were seen. At the highest dose in dogs, at histopathological examination of tissues post mortem there was congestion and necrosis in the liver, together with fatty change and, in the spleen, evidence of extramedullary haematopoiesis. Fatty change in the myocardium and cloudy swelling of the renal tubular cells were also

observed. The NOAEL for the study in mice was 400 ppm (equal to 90 mg/kg bw per day) on the basis of prolonged prothrombin and partial thromboplastin times at higher dietary concentrations. The NOAEL for the study in rats was 400 ppm (equal to 25.3 mg/kg bw per day) on the basis of clinical chemistry changes observed at the next highest dietary concentration. In the study in dogs, the NOAEL was 300 ppm (equal to 12.0 mg/kg bw per day) on the basis of clinical effects observed at higher dietary concentrations. Three deaths were observed at the highest dose in weeks 11 and 12 of the study. In a 1-year study in dogs, clinical signs (emaciation, dehydration, hyperaemia, alopecia and diarrhoea, which was occasionally bloody) were seen at the highest dietary concentration. The NOAEL for the study was 400 ppm (equal to 13.1 mg/kg bw per day) on the basis of clinical signs, weight loss and anaemia at the highest dietary concentration. It was not considered appropriate to set an ARfD on the clinical signs, reduced body weight or haematological changes occurring in dogs, since significant clinical effects were not seen early in these two studies.

Three studies of developmental toxicity in rats treated by gavage (two studies) or by dietary administration (one study) were evaluated by the Meeting. In the earlier study of rats treated by gavage, neither maternal nor fetal toxicity was seen at any dose; the NOAEL for both maternal and fetal toxicity was thus 200 mg/kg bw per day, the highest dose tested. In the later study in rats treated by gavage, in which higher doses were administered, the NOAEL was 100 mg/kg bw per day on the basis of maternal toxicity (decreased food consumption) and fetal toxicity (postimplantation loss, reduced fetal weight and incompletely ossified fetal skeletons). In the study of developmental toxicity in rats given diets containing bentazone, the NOAEL for maternal toxicity was 4000 ppm (equal to 324 mg/kg per day) on the basis of decreased weight gain and food consumption at 8000 ppm. The NOAEL for fetal toxicity was also 4000 ppm (equal to 324 mg/kg bw per day) on the basis of decreased fetal weights and fetal liver petechiae at 8000 ppm. Bentazone was not found to be teratogenic in any of the studies of developmental toxicity in rats. The Meeting assessed two studies of developmental toxicity in rabbits. In one study, the NOAEL for maternal and fetal toxicity was 150 mg/kg bw per day, the highest dose tested; neither maternal nor fetal toxicity was observed at any dose. In the second study, in which bentazone was administered at higher doses than in the earlier study, the NOAEL for maternal toxicity was 150 mg/kg bw per day on the basis of reduction in maternal food consumption at 375 mg/kg bw per day. Postimplantation losses were increased at 375 mg/kg bw per day and there was total implantation loss in one dam. Bentazone was not teratogenic in either study of developmental toxicity in rabbits.

Two case reports of fatal self-poisoning in humans were characterized by vomiting, diarrhoea, drowsiness and death from cardiac arrest.

Toxicological evaluation

The Meeting concluded that the establishment of an ARfD was unnecessary.

Estimate of acute reference dose

Unnecessary

Studies that would provide information useful for continued evaluation of the compound

Further observations in humans

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	13-week study of toxicity[a]	Toxicity	400 ppm, equal to 25.3 mg/kg bw per day	1200 ppm, equal to 77.8 mg/kg bw per day
	Two-generation study of reproductive toxicity[a,b]	Parental toxicity	800 ppm, equal to 62 mg/kg bw per day	3200 ppm, approximately equal to 250 mg/kg bw per day
		Reproductive toxicity	200 ppm, equal to 15 mg/kg bw per day	800 ppm, equal to 62 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	100 mg/kg bw per day	250 mg/kg bw per day
		Embryo and feto-toxicity	100 mg/kg bw per day	250 mg/kg bw per day
Rabbit	Developmental toxicity[c]	Maternal toxicity	150 mg/kg bw per day	375 mg/kg bw per day
		Embryo and feto-toxicity	150 mg/kg bw per day	375 mg/kg bw per day
Dog	13-week study of toxicity[c,d]	Toxicity	300 ppm, equal to 12.0 mg/kg bw per day	1000 ppm, equal to 39.6 mg/kg bw per day
Dog	1-year study of toxicity	Clinical signs	400 ppm, equal to 13.1 mg/kg bw per day (1600 ppm, equal to 52.3 mg/kg bw per day for ARfD assessment)	1600 ppm, equal to 52.3 mg/kg bw per day

[a] Diet
[b] Evaluated previously (Annex 1, reference 62)
[c] Gavage
[d] Effects evident at early time-points

References

Allen, T.R., Frei, T., Luetkemeier, H., Vogel, O., Terrier, C., Vogel, W. & Wilson, J. (1989) 52-Week oral (feeding) study with bentazone technical (ZST No. 86/48) in the dog. Unpublished report No. 89/0049 from RCC research and consulting company AG and RCC Umweltchemie AG, Itingen, Switzerland and EPS Experimental Pathology Services (UK) Ltd, Hereford, England. Submitted to WHO by BASF. GLP: CFR 48, 230 (1983), MAFF (Japan) 59 NohSan 3850 (1984), Swiss GLP Guidelines (1986), OECD 1981. Guidelines EPA chronic toxicity studies, pesticide assessment guidelines, subdivision F, Hazard evaluation: human and domestic animals, 83-1 (1982), OECD 452, MAFF (Japan) 59 NohSan 4200.

Anonymous (1981) Thirty-day oral toxicity study of bentazone in mice. Unpublished report No. 81/10239 from Nippon Institute for Biological Science, Tokyo, Japan. Submitted to WHO by BASF. GLP status not given in report.

Becker, H., Frei, D., Vogel, W. & Terrier, C. (1986) Embryotoxicity (including teratogenicity) study with bentazon technical in the rat. Unpublished report No. 86/421 from RCC research and consulting company AG. Itingen, Switzerland. Submitted to WHO by BASF. GLP: CFR 48, 230, MAFF (Japan) 59 NohSan 3850, Swiss GLP Guidelines. Guidelines USEPA 83-3 (1982), OECD 414 (1981), MAFF (Japan) 4200 (1985).

Becker, H., Muller, E., Vogel, W. & Terrier, C. (1987) Embryotoxicity (including teratogenicity) study in the rabbit. Unpublished report No. 87/058 from RCC research and consulting company AG, Itingen, Switzerland. Submitted to WHO by BASF. GLP: USEPA CFR 48, 230 (1983), MAFF (Japan) 59 NohSan 3850 (1984), Swiss GLP Guidelines (1986). Guidelines USEPA CFR 43 163 83-3 (1982), OECD 414 (1981), MAFF (Japan) 59 NohSan 4200 (1985).

Hofman, H.T. & Merkle, J. (1978a) Investigation to determine the prenatal toxicity of 3-isopropyl-2,1,3-benzothiadiazin-4-one2,2-dioxide on rats. Unpublished report No. 78/039 from BASF Gewerbehygiene und Toxikologie, Ludwigshafen, Germany. Submitted to WHO by BASF. Pre-GLP. Guidelines FDA, 1966; ABPI, 1975.

Hofmann, H.T. & Merkle, J. (1978b) Study to determine the prenatal toxicity of 3-(1-methylethyl)-1H-2,1,3-benzothiadiazin-4(3H)-on-2,2-dioxide in rabbits. Unpublished report No. 84/048 from BASF Toxicology Dept, Ludwigshafen, Germany. Submitted to WHO by BASF. Pre-GLP. Guidelines FDA, 1966; ABPI, 1975.

Itabashi, M., Inoue, T., Takehara, K. & Tajima, M. (1982) Teratogenicity study of bentazon, reg. No. 51 929 (ZNT no 81/273) in rats by dietary administration. Unpublished report No 84/066 from Nippon Institute for Biological Science, Tokyo, Japan. Submitted to WHO by BASF. GLP: FDA 1978. Guideline None mentioned.

Leuschner, F., Leuschner, A., Schwerdtfeger, W. & Otto, H. (1970) 13-Week toxicity of 3-isopropyl-1H-2,1,3-benzothiadiazin-4(3H)-one-2,2-dioxide (hereafter referred to as XIX/410) to beagles when administered in

the food. Unpublished report No. 70/009 from Pharmacology and toxicology laboratory, Ludwigshafen, Germany. Submitted to WHO by BASF.

Müller, I.B., Petersen, H.W., Johansen, S.S. & Theilade, P. (2003) Fatal overdose of the herbicide bentazone. *Forensic Sci. Int*., **135**, 235–236.

Tennekes, H., Horst, K., Leutkemeier, H., Wilson, J., Vogel, W. & Terrier, C. (1987) 13 week oral toxicity (feeding) study with bentazon technical (ZNT No. 86/48). RCC research and consulting company AG and RCC Umweltchemie AG, Itingen, Switzerland. Submitted to WHO by BASF. GLP: USEPA, CFR 48, 230, Swiss GLP Guidelines 1982, OECD 1981. Guidelines EPA Sub-chronic oral toxicity, Hazard evaluation, human and domestic animals, 83-1 (1982), OECD 408 (1981).

Turcant, A., Harry, P., Cailleus, A., Puech, M., Bruhat, C., Vicq, N., le Bouil, A. & Allain, P. (2003) Fatal acute poisoning by bentazone. *J. Anal. Toxicol*., **27**, 113–117.

CAPTAN (addendum)

First draft prepared by
G. Wolterink and M.T.M. van Raaij
Centre For Substances and Integrated Risk Assessment
National Institute of Public Health and the Environment, Bilthoven, Netherlands

Explanation

Captan is a fungicide used for the control of fungal diseases in crops. The Meeting evaluated captan in 1963, 1965, 1969, 1973, 1978, 1982, 1984, 1990 and 1995. Toxicological monographs were prepared in 1963, 1965 and 1969, and addenda to the monographs were prepared in 1973, 1977, 1978, 1982, 1984, 1990 and 1995. In 1984, an acceptable daily intake (ADI) of 0–0.1 mg/kg bw was established on the basis of a no-observed-adverse-effect level (NOAEL) of 12.5 mg/kg bw per day in studies of reproductive toxicity in rats and monkeys. The present Meeting considered the requirement for an acute reference dose (ARfD), on the basis of data from the previous evaluations and from new studies.

Evaluation for acute reference dose

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Mice

Two studies were performed to establish to what extent captan and its metabolites are present in the duodenum and other regions of the gastrointestinal tract of mice treated orally with captan. Quality assurance (QA) and good laboratory practice (GLP) statements were provided.

In the first study, groups of male CD1 mice were fed control diet (six mice per group) or diet containing captan at a concentration of 400 or 3000 ppm, equivalent to 60 or 450 mg/kg bw (30 mice per group), for 8 days, the acclimatization period. This was included because high concentrations of captan in the diet have been found to produce a short-term reduction in food intake. The diets containing captan were then replaced by diets containing [1,2 ^{14}C]cyclohexene-labelled captan, (purity, 99.1%) at the start of the 12 h dark phase.

At 6, 12, 18, 24 and 30 h after introduction of the radiolabelled diets, six mice per group were sacrificed, samples of blood and urine (from those animals with full bladders) were taken, and the gastrointestinal tract was removed. All samples were stored until analysis by radiochemistry ($n = 4$) or high-performance liquid chromatography (HPLC) ($n = 2$). Before analysis, the stomach was removed, the duodenum was cut into two 5 cm sections and the remaining gastrointestinal tract up to the caecum was cut into 10 cm sections. The caecum was analysed separately and the remaining sections of colon and rectum to anus were analysed together.

At each time-point, the amount of radiolabel measured in the gastrointestinal tract was low in comparison with the total amount ingested. At 3000 ppm, the recovery from sections including the duodenum to the anus ranged from 12.7% of the ingested dose after 6 h to 2.2% after 30 h. During the dark periods (after 6–12 h and after 24–30 h), radiolabel was mainly found in the stomach and in the caecum. In the daylight period (after 18–24 h), less radiolabel was present in the stomach, suggesting reduced food uptake, while no decline in radiolabel was observed in the caecum (probably owing to the concentration of intestinal material before excretion as faeces). During the 30 h experimental period, the concentration of radiolabelled material in the duodenum (the first 10 cm of the intestine) and the following 30 cm of the intestine remained constant, indicating a steady-state concentration of captan or metabolites during this time. At the lower dose of 400 ppm, the distribution of radiolabel along the gastrointestinal tract was found to be similar to that at 3000 ppm. The amount of material present in the gastrointestinal tract from the duodenum to the caecum was about sevenfold higher in animals in the group receiving the highest dose than in the group receiving the lowest dose, i.e. the amount of material present increased in proportion to the increase in dose (Provan & Eyton-Jones, 1996).

In the second study, the acclimatization and dosing protocols were identical to those used in the first study. At 12 h and 24 h after introduction of the diets containing radiolabelled captan, five males per group were sacrificed, and blood samples were taken. Stomach and duodenum sections (0–5 and 5–10 cm) were separated from their contents, and tissue from three mice per group was analysed for total radiolabel, while tissue from two mice per group was analysed for captan and metabolites.

The amount of radiolabelled material present in the duodenum of mice from both studies was similar, with about 0.006 µmol/5 cm section and 0.05 µmol/5 cm section at 400 and 3000 ppm respectively. Most of the radiolabel in the stomach and duodenum appeared to be associated with the stomach contents and not with the tissue. The parent compound captan could not be detected in the duodenum. In the stomach, captan was found in only three (out of four) mice at 3000 ppm (after 12 and 24 h). In the duodenum of these animals a small amount of metabolite of captan was detected. The only metabolite that was identified in the gastrointestinal tract was 1,2,3,6-tetrahydrophthalimide (THPI) which was found in the stomach. Blood samples contained small amounts of radiolabel. No captan was detected by HPLC in blood or urine samples. Metabolites were present in the urine in relatively large amounts compared with those in the gastrointestinal tract, with up to seven peaks being detected. The metabolites in urine were not further identified.

From these studies it was concluded that the captan molecule is largely degraded in the stomach before reaching the duodenum and consequently cannot be detected in duodenal contents, blood or urine (Provan & Eyton-Jones, 1996).

1.2 Biotransformation

Humans (in vitro)

^{14}C-Labelled captan (radiochemical purity, 80.9%) was incubated with whole human blood in vitro at 37 °C. After incubation for times ranging from 0 to 22 s, the reaction was terminated by addition of phosphoric acid and acetone. Degradation of captan was measured. GLP and QA statements were provided.

Captan was degraded rapidly to THPI in whole blood, with a calculated half-life of 4 s. THPI was not significantly degraded to other compounds within the short period of time studied (Gordon & Williams, 1999).

In a study from the published literature, the degradation of captan (purity, 79.9%) during incubation with human blood was investigated. Captan at a concentration of about 1 µg/ml was mixed with blood at 37 °C. At various time-points ranging from 0 to 31 s the reaction was terminated by adding phosphoric acid and acetone. Degradation of captan and formation of THPI was measured.

Captan was metabolized rapidly to THPI. The calculated half-life was 0.97 s. Mass spectrometry revealed that THPI was the only degradation product (Gordon et al., 2001).

2. Toxicological studies

2.1 Short-term studies of toxicity

Groups of 25 male CD1 mice received food containing captan (purity, 89.4%) at a concentration of 3000 ppm for 28 days. The actual consumption of captan increased from about 440 mg/kg bw per day on the first 2 days of treatment to about 700 mg/kg bw per day from day 7 to day 28. Since it was anticipated that there would be reduced food consumption, a pair-fed control group was fed the average amount of food consumed by the mice fed with captan. Clinical observations, body weight and food consumption were measured after 1, 3, 7, 14 and 28 days of treatment for five males per group, and the small intestine and stomach were also examined histopathologically at these time-points.

One mouse in the treated group and one mouse in the pair-fed group were killed on day 4 due to excessive body-weight loss. Among the mice fed with captan and the pair-fed mice, body weights were significantly reduced. These groups showed a body-weight loss of 2–3% at the end of the study, while a body-weight gain of 11% was observed in the control group. Food consumption was reduced by 37% on the first day of treatment and remained lower than that of controls throughout the first week of treatment. Food consumption was slightly reduced from weeks 2 to 4.

In mice fed with captan at a concentration of 3000 ppm for one day, no treatment-related abnormalities were observed in the duodenum or any other of the examined tissues. After 3 days of treatment, the duodenum showed crypt cell hyperplasia, shortening of the villi and a general disorganization of the villus enterocytes. In addition, immature cells were seen at the villus tips in all mice fed with captan from day 7 to day 28. In one out of five mice observed at days 3 and 7, gastritis in the glandular portion of the stomach was observed. On day 28, one mouse displayed a focal parakeratosis in the stomach. No treatment-related changes were observed in the jejenum or ileum. It was concluded that

crypt cell hyperplasia occurs rapidly after exposure to captan at 3000 ppm. Changes in the villus or crypt cell population preceding the hyperplasia were not observable by routine histological methodology (Tinston, 1996).

In a range-finding study for a 1-year study in dogs, groups of two male and two female beagle dogs were given gelatin capsules containing captan at a dose of 0, 30, 100, 300, 600 or 1000 mg/kg bw per day for 28 days. The dogs were observed daily for clinical signs. Body weights and food consumption were determined weekly. At termination, haematological and clinical biochemical parameters were determined, liver and kidneys were weighed, and microscopic examination was performed on duodenum, kidney and liver of animals from the control group and from the group receiving captan at a dose of 1000 mg/kg bw per day.

Dose-related emesis, decreased body-weight gain and decreased food consumption were observed at all doses, except in the control group. Slight, statistically non-significant changes in clinical biochemical parameters were mainly observed at the two higher doses. These changes were probably related to emesis, reduced food consumption and body-weight loss. No toxicologically relevant effects on haematological parameters were observed. One male in the group receiving the highest dose had fatty changes in the liver and collecting tubules of the kidney. The toxicological significance of these findings is not clear. The histopathology of the duodenum was reported to be within normal limits (Blair, 1987).

2.2 Developmental toxicity

Hamsters

In a study from the published literature, the teratogenic effects of a number of derivatives of phthalimide, including captan, were tested in groups of two to eight pregnant golden hamsters. Captan was administered as a single dose at 200, 300, 400, 500, 600, 750 or 1000 mg/kg bw on day 7 or 8 of gestation, or daily at a dose of 100, 200, 300 or 500 mg/kg bw per day from days 6 to 10 of gestation. Groups of dams serving as controls received either no treatment (n = 43) or carboxymethyl cellulose at a dose of 10 ml/kg bw (n = 99). The animals were killed and examined on day 15 of gestation. In the study report, the doses for the group treated with repeated doses on days 6 to 10 of gestation are expressed as total dose. It is assumed that this total dose was administered evenly over the 5 days of treatment, with one treatment per day.

In the groups treated with captan, maternal mortality occurred at single doses of ≥600 mg/kg bw and at repeated doses of ≥300 mg/kg bw per day. In the group treated on day 7 with a single dose of captan of ≥750 mg/kg bw, increases in the incidence of exencephaly were observed. There was no dose–response relationship. One case of exencephaly (out of 67 fetuses observed) was reported in animals treated with a single dose of 300 mg/kg bw on day 8 of gestation. At 500 mg/kg bw, there was one case (out of 58 fetuses observed) of exencephaly and three cases of cranial "pimples" (neural tissue not protruding). Significantly increased incidences of fused ribs were observed in fetuses of dams treated with a single dose of captan on days 7 or 8 of gestation. The lowest dose at which this effect was observed was at 300 mg/kg bw. Occasionally other skeletal malformations were observed in animals treated with single doses of captan on day 7 or day 8 of gestation. The only reported skeletal abnormality in the groups receiving repeated doses was one case of fused ribs in a fetus at 500 mg/kg bw per day. The study did not comply with GLP (Robens, 1970).

The Meeting noted that mortality in the groups treated with repeated doses of captan appeared to be related to dose. In the groups receiving captan as single doses, the relationship between mortality and dose was less clear. For the observed and skeletal effects of captan in the golden hamster, no clear dose–response relationship was observed. This may be related to the small numbers of litters and fetuses in the treated groups. Nevertheless, the incidences of exencephaly on day 7 and fused ribs on days 7 or 8 were clearly above control values. Apart from the data on mortality, and the statement that diarrhoea occurred in many of the treated dams, no information about maternal toxicity was detailed in the paper. The Meeting considered that the mortality and developmental toxicity observed in this study were toxicologically relevant. Owing to the variability of the data, NOAELs for the maternal and fetal effects of captan could not be established. The Meeting noted that this study has major limitations (e.g. small number of animals per dose, limited reporting of the data) and is therefore of limited value. It does, however, suggest that developmental effects may occur after a single exposure to captan, albeit at maternally toxic doses.

Rats

In a study of developmental toxicity performed according to OECD test guideline 415, groups of 22 pregnant ChR-CD rats were treated with captan (purity, 91%) at a dose of 0, 18, 90 or 450 mg/kg bw per day by oral gavage in 0.5% carboxymethylcellulose containing 0.05% acetic acid from day 6 to day 15 of gestation. The dams were killed on day 20 of gestation, fetuses were removed and dams and fetuses were examined to determine maternal toxicity, and reproductive and developmental effects. GLP and QA statements were provided.

In dams in the group receiving the highest dose, food consumption was decreased by 47% during the first 3 days of treatment and by 17% throughout the rest of the treatment period. Body-weight gain was decreased in dams in this group throughout the treatment period (controls: +45 g, highest dose, +23 g). Reduction was most marked during the first 3 days of treatment. In the dams receiving the intermediate dose, food intake was reduced by 18% from days 7 to 9 of gestation, and body-weight gain was reduced on days 7 and 8 of gestation (controls, +3 and +9 g, intermediate dose, −5 and −1 g). Pup viability was not affected by the treatment. An increased pre-implantation loss was observed at the intermediate dose, but not at the highest dose. Fetal weight was decreased at the highest dose. No treatment-related malformations were observed in any of the treated groups. A slight treatment-related increased incidence of skeletal variants (14th lumbar rib, incompletely fused vertibral hemicentra, reduced ossification of the pubic bones) was observed in fetuses in the group receiving the highest dose. On the basis of the decreases in body weight and food consumption at the intermediate and highest doses, the NOAEL for maternal toxicity was 18 mg/kg bw per day. On the basis of the decrease in fetal body weight and increase in incidence of skeletal variations observed at 450 mg/kg bw per day, the NOAEL for embryo/fetotoxicity was 90 mg/kg bw per day (Rubin, 1987).

Rabbits

Groups of 15 pregnant New Zealand White rabbits received captan (dissolved in 0.5% sodium carboxymethyl cellulose) at a dose of 0, 6, 12, 25, or 60 mg/kg bw per day by gavage from day 6 to day 28 of gestation. Does were killed on day 29, litter values were determined and fetuses were examined for external, visceral and skeletal abnormalities. Body weights were measured on days 6, 10, 14, 18, 22, 26 and 29. QA and GLP statements were provided.

The incidences of animals that were found dead or were killed were 2, 1, 2, 2 and 1 in the groups receiving captan at a dose of 0, 6, 12, 25 and 60 mg/kg bw per day, respectively. The deaths of a single animal in each of the groups receiving 6, 25 and 60 mg/kg bw per day were considered to be a consequence of intubation errors. The other deaths were not considered to be related to treatment. The numbers of animals found not to be pregnant were 1, 0, 0, 1 and 6, and the incidences of abortions were 0, 1, 2, 3 and 0 in the groups receiving 0, 6, 12, 25 and 60 mg/kg bw per day, respectively. Consequently, at termination of the study, the number of animals with live fetuses were 12, 13, 11, 9 and 8, respectively. The reduction in pregnancy rate at the highest dose was probably not related to treatment. Over the total treatment period, body-weight gains at 0, 6, 12, 25 and 60 mg/kg bw per day were 521, 529, 426, 434 and 408 g, respectively. Reductions in body-weight gain at 25 and 60 mg/kg bw per day were most marked during days 6 to 10 of gestation (+72 and −9 g respectively, versus +111 g in control animals). Body-weight gain at 12 mg/kg bw was retarded from day 18 onwards. At the highest dose, slight reductions in fetal weight (8%) and crown–rump length (4%) were observed. Incidences of major malformations, minor anomalies (visceral and skeletal) and skeletal variants were not significantly affected by treatment.

On the basis of effects on maternal body weight during the initial phase of treatment, the NOAEL was 12 mg/kg bw per day. On the basis of the effects on fetal body weight, the NOAEL for embryo/fetotoxicity was 25 mg/kg bw per day (Palmer et al., 1981).

The Meeting noted that body weight was not measured on a daily basis. Therefore any transient effects on body weight cannot be established.

Groups of 20 female New Zealand White rabbits received captan (in corn oil) at a daily dose of 0, 10, 30 or 100 mg/kg bw by oral gavage from day 7 to day 19 (inclusive) of gestation. The day of insemination was designated day 1 of gestation. On day 30, the animals were sacrificed and maternal and developmental toxicity were examined. The study was performed according to OECD test guideline 414.

Body-weight gain and food consumption were reduced in dams at 30 and 100 mg/kg bw per day. Body-weight gain during days 7 to 19 of gestation was 238, 205, 57 and −159 g at 0, 10, 30 and 100 mg/kg bw per day, respectively. Reduction in body-weight gain was most marked during days 7 to 10 of treatment (16, 40, −68 and −143 g at 0, 10, 30 and 100 mg/kg bw per day, respectively). Food consumption was correspondingly reduced. One female at the highest dose was killed on day 19 after signs of abortion. In dams in all treated groups, a dose-dependent increased incidence of few or no faeces and a slightly increased incidence of diarrhoea were observed. In one and three dams at the intermediate and highest dose, blood was observed in the urine. On the basis of reduced body-weight gain and reduced food consumption, the NOAEL for maternal toxicity was 10 mg/kg bw per day.

Compared with the control group, an increased incidence of postimplantation loss was observed at the highest dose (control, 7%, highest dose, 23%), and the incidences of both early (3% versus 11%) and late intrauterine deaths (4% versus 12%) were increased. The statistically non-significant increase in late intrauterine deaths at the intermediate dose was within the range for historical controls. Mean fetal body weight was decreased by 17% at 100 mg/kg bw per day, which was reflected in a decreased mean gravid uterus weight. At the two higher doses, the incidence of abnormal fetuses was increased. The number of fetuses with one or more major abnormalities at 0, 10, 30 and 100 mg/kg bw per day was,

respectively, 1, 0, 2 and 8. Eight fetuses (out of 86) from five different litters at 100 mg/kg bw per day had at least one major abnormality compared with only one fetus (out of 102) in the control group. Four of the eight affected fetuses at 100 mg/kg bw per day were in the same litter. The type and incidence of major defects at 100 mg/kg bw per day were: gross torso malformations, one; encephalocoele/open eyes/gros malformation of skull, one; mid brain ventricles extremely dilated/cebocephaly, one; maxillae fused, two; pulmonary artery extremely reduced, one; 11th rib and thoric arch absent, one; omphalocoele, one; forepaw extremely flexed, two; and pollex absent–bilateral, one. Two fetuses (out of 75) from two different litters at 30 mg/kg bw per day had one or multiple major abnormalities. The type and incidence of major defects in these two animals at 30 ppm were: microphthalmia, one; fused mandibles, one; pulmonary artery extremely enlarged, one; subclavian artery absent, one; and forepaw extremely flexed, one. These multiple malformations observed in two fetuses in the group receiving the intermediate dose were considered to be incidental. One fetus in the control group had an extremely enlarged aorta. In fetuses in the groups receiving the intermediate and highest doses; an increased incidence of cysts on the liver was found (one, one, five and six at 0, 10, 30 and 100 mg/kg bw per day, respectively). Also in fetuses in the groups receiving the intermediate and highest doses, increases in the incidences of a number of skeletal variations were observed. Incidences of these variations at 0, 10, 30 and 100 mg/kg bw per day were: extra 13th rib with normal length, 61%, 52%, 77% and 78%; 27 pre-sacral vertebrae, 21%, 12%, 57% and 46%, asymmetrical development of 1st and 2nd sacral vertebrae, 2%, 1%, 5% and 7%. At the intermediate and highest doses, slight increases in the incidences of the following variations were also observed: 4th, 5th, 6th and 7th lumbar transverse processes partially ossified; and 3rd lumbar transverse processes partially ossified; odontoid partially ossified. In addition, at the highest dose, the incidence of partially ossified 2nd lumbar transverse processes was increased. Also at the highest dose, the mean manus score was significantly increased, which reflects reduced ossification. On the basis of the increased incidences of variations in fetuses at 30 mg/kg bw per day, the NOAEL for embryo/fetotoxicity was 10 mg/kg bw (Tinston, 1991).

Four groups of mated HY/CR New Zealand White rabbits (aged 4–5 months) were given captan (active ingredient, 91%) at a dose of 0 (vehicle), 10, 40 or 160 mg/kg bw per day by oral gavage in 0.5% carboxymethylcellulose containing 0.05% acetic acid, from day 7 to day 19 inclusive post coitum. The numbers of animals in the groups were 18, 14, 15 and 16, respectively. A reduced food intake was observed at the highest dose throughout the treatment period. At the intermediate dose, food intake was reduced during days 15 to 19 of treatment. Over the treatment period, a marked effect on body weight was seen at the highest dose (control, +144 g, highest dose, −124 g during treatment). A slight effect on body weight (−7 g) was observed in dams in the group receiving the intermediate dose group after the first day of treatment. Adverse fetal responses to treatment consisted of increased fetal death (postimplantation loss) at 160 mg/kg bw per day, with one case of abortion after death of all fetuses in the litter and one case of total fetal death discovered at terminal necropsy. In addition, an increased frequency of minor skeletal variations was recorded in fetuses at the highest dose. Fetuses of dams dosed at 40 or 10 mg/kg bw per day were not affected. Captan did not show a potential for teratogenicity in the New Zealand White rabbit at the highest dose, and did not cause adverse fetal effects in the absence of maternal toxicity under the conditions of this study. On the basis of the effects of captan on food consumption at 40 mg/kg bw per day, the NOAEL for maternal toxicity was 10 mg/kg bw per day. On the basis of the increase in postimplantation loss and the increase in incidence of minor skeletal variations at 160 mg/kg bw per day, the NOAEL for embryo/fetotoxicity was 40 mg/kg bw per day (Rubin & Nyska, 1987).

3. Observations in humans

Captan has caused allergic dermatitis and eye irritation in man (Maddy et al., 1990; WHO, 1990).

The effects of ingestion of captan were described in a concise case report. In an attempted suicide, a woman aged 17 years (body weight not reported) ingested 7.5 g of Captan 50 WP, which is a suspension of captan in a ratio of 50% mixed with water. The woman complained of headache, nausea, weakness, numbness of upper limbs, and substernal pain. These symptoms started 3 h after ingestion. Cardiac, pulmonary, abdominal and neurological examinations were normal. Haematology revealed slight elevations in leukocyte count (11 200/mm^3), creatine kinase (309 U/l, normal up to 43 U/l) and aspartate aminotransferase (67 U/l, normal up to 29 U/l). Electrocardiogram (ECG) showed inversion of T-waves in the III and aVF leads and echocardiogram was normal with an ejection fraction of 67%. After 72 h, all biochemical abnormalities resolved (Chodorowski & Anand, 2003).

Comments

In the evaluation of captan by the JMPR in 1995, it is reported that in mice treated orally with captan, the captan molecule is largely degraded to THPI and thiophosgene (via thiocarbonyl chloride) in the stomach before reaching the duodenum. No captan was detected in the blood or urine. Studies of metabolism in vitro with human blood revealed that captan is rapidly degraded to THPI, with a calculated half-life of 1–4 s. Thiophosgene is detoxified by reaction with, e.g. cysteine or glutathione, and is ultimately rapidly excreted.

The acute oral toxicity of captan in rats is low (LD_{50}, >5000 mg/kg bw). Mice fed diets containing captan at a concentration of 3000 ppm, equal to 440 mg/kg bw per day, for 28 days showed an initial reduction in food consumption of about 37%. Food consumption gradually recovered over the first week of treatment, although it remained lower that that of controls throughout the 4-week treatment period. After 1 day, no treatment-related macroscopic and microscopic changes were observed in the duodenum or any other tissue examined. From day 3 onwards, the duodenum showed crypt cell hyperplasia, shortening of villi and a general disorganization of the villus enterocytes. From day 7 onward, immature cells were seen at the villus tips.

In a 28-day range-finding study in which dogs were given captan at doses of 30 to 1000 mg/kg bw per day, dose-related emesis, reduced body-weight gain and food consumption were observed in all treatment groups. No other clinical signs were observed. Haematological parameters and histopathology of the duodenum were within normal limits.

In a study from the published literature, the teratogenic effects of a number of phthalimide derivatives, including captan, were tested in pregnant golden hamsters. The Meeting noted that this study had major limitations (e.g. small number of animals per dose, limited reporting of the data) and is therefore of limited value. It does, however, suggest that developmental effects may occur after a single exposure to captan, albeit at maternally toxic doses.

In a study of developmental toxicity in rats treated by gavage, captan was not teratogenic. The NOAEL for maternal toxicity was 18 mg/kg bw per day on the basis of a reduc-

tion in body weight and food consumption. The NOAEL for offspring toxicity was 90 mg/kg bw per day on the basis of the reduction in fetal body weight and an increased incidence of skeletal variations.

In a study in rabbits treated by gavage, the NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of a markedly reduced body-weight gain and reduced food consumption at 30 mg/kg bw per day. The NOAEL for embryo/fetotoxicity was 10 mg/kg bw per day on the basis of increases in skeletal variations at 30 and 100 mg/kg bw per day. At 100 mg/kg bw per day, increased incidences of early and late intrauterine deaths were observed, as were increased incidences of several malformations. The NOAEL for these effects was 30 mg/kg bw per day. Multiple malformations observed in two fetuses in the group receiving the intermediate dose were considered to be incidental. In another study in rabbits treated by gavage, the NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of reduced body-weight gain and food consumption at 40 mg/kg bw per day. On the basis of the increase in postimplantation losses and the increase in incidence of minor skeletal variations at 160 mg/kg bw per day, the NOAEL for embryo/fetotoxicity was 40 mg/kg bw per day. In a third study in rabbits treated by gavage, the NOAEL for maternal toxicity was 12 mg/kg bw per day on the basis of reductions in body-weight gain during the initial phase of treatment. The NOAEL for embryo/fetotoxicity was 25 mg/kg bw per day on the basis of a reduction in fetal body weight at 60 mg/kg bw per day. The Meeting considered that maternal toxicity and the associated increases in skeletal variations and fetal body-weight reductions observed were likely to be caused by high local concentrations of captan produced by administration by gavage, and were not relevant to dietary exposure.

While few data on humans are available, captan is known to have caused allergic dermatitis and eye irritation in humans. After ingesting 7.5 g of Captan 50 WP, which is a suspension of captan mixed with water (ratio, 50%), a woman aged 17 years (body weight not reported) experienced some clinical signs, which started 3 h after ingestion and recovered within 72 h. Assuming a body weight of 50–60 kg, this intake equates to a dose of 62.5–75 mg/kg bw.

Toxicological evaluation

Other than developmental effects, captan produced no toxicological effects that might be considered to be a consequence of acute exposure. The Meeting concluded that it was not necessary to establish an ARfD for the general population, including children aged 1–6 years, for whom separate data on dietary intake are available. The Meeting concluded that it might be necessary to establish an ARfD to protect the embryo or fetus from possible effects in utero. Such an ARfD would apply to women of childbearing age.

The maternal toxicity and associated increases in skeletal variations and fetal body-weight reductions observed in studies of developmental toxicity in rabbits are likely to be caused by high local concentrations of captan and are not considered to be relevant to dietary exposure. However, the observed intrauterine deaths and fetal malformations could not, with confidence, be attributed to maternal toxicity.

The Meeting concluded that the database was insufficient (in particular, with regard to the absence of studies on the developmental effects of THPI to establish the mode of action by which the increased incidences of intrauterine deaths and of fetuses with

malformations, observed at 100 mg/kg bw per day (NOAEL, 30 mg/kg bw per day) in rabbits, were induced. As a consequence, their relevance for deriving an ARfD could not be dismissed. Therefore the Meeting established an ARfD of 0.3 mg/kg bw, based on a NOAEL of 30 mg/kg bw per day for increased incidences of intrauterine deaths and malformations at 100 mg/kg bw per day in the study in rabbits and a safety factor of 100. The use of a safety factor of 100 was considered to be conservative; although the mode of action by which the developmental effects were induced is uncertain, they are possibly secondary to maternal toxicity. The ARfD also covers the effects observed in the case report in humans. The Meeting noted that it might be possible to refine the ARfD using the results of an appropriately designed study.

Estimate of acute reference dose

0.3 mg/kg bw for women of childbearing age

Unnecessary for the general population

References

Blair, M. (1987) Four-week oral range-finding study in beagle dogs with captan. Unpublished report No. IRDC 153-197 from ICI Americas. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

Chodorowski, Z. & Anand, J.S. (2003) Acute oral suicide intoxication with captan—a case report. *J. Toxicology*, **41**, 603.

Gordon, E.B. & Williams, M. (1999) The stability of captan and folpet in whole blood. Unpublished report No. 10238 from Horizon Laboratories, Inc. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

Gordon, E.B., Ehrlich, T., Mobley, S. & Williams, M. (2001) Measurement of the reaction between the fungicides captan or folpet and blood thiols. *Toxicol. Methods*, **11**, 209–223.

Maddy, K.T., Edmiston, S. & Richmond, D. (1990) Illness, injuries and deaths from pesticide exposures in California 1949–1988. *Rev. Environ. Contam. Toxicol*., **114**, 57–123.

Palmer, A.K., Barton, S.J. & Clark, R. (1981) Effect of technical captan on pregnancy of the New Zealand white rabbit. Unpublished report No. CHR/15/8114 from Huntingdon Research Centre, England. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

Provan, W.M. & Eyton-Jones, H. (1996) The bioavailability of captan to the duodenum of CD-1 mice following dietary administration. Unpublished report No. CTL/R/1260 from Zeneca, USA. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

Robens, J.F. (1970) Teratogenic activity of several phthalimide derivates in the golden hamster. *Toxicol. Appl. Pharmacol.*, **16**, 24–34.

Rubin, Y. (1987) Captan teratology study in the rat. Unpublished report No. MAK/097/CAP from Life Science Research Israel Ltd. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

Rubin, Y. & Nyska, A. (1987) Captan teratology study in the rabbit. Unpublished report No. MAK/099/CAP from Life Science Research Israel Ltd. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

Tinston, D.J (1991) Captan: teratogenicity study in the rabbit. Unpublished report No. CTL/P/3039 from ICI Americas. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

Tinston, D.J. (1996) Captan: A time course study of induced changes in the small intestine and stomach of the male CD1 mouse. Unpublished report No. CTL/P/4893 from Zeneca Agrochemicals UK. Submitted to WHO by Makhteshim Chemical Works, Beer-Sheva, Israel.

WHO (1990) *Captan. Health and safety guide No. 50*. Geneva, International Programme on Chemical Safety.

DIMETHIPIN (addendum)

First draft prepared by
G. Wolterink and M.T.M. van Raaij
Centre For Substances and Integrated Risk Assessment, National Institute of Public Health and the Environment, Bilthoven, Netherlands

Explanation

The 1999 JMPR established an acute reference dose (ARfD) for dimethipin of 0.02 mg/kg bw, based on a no-observed-adverse-effect level (NOAEL) of 20 mg/kg bw per day and a lowest-observed-adverse-effect level (LOAEL) of 40 mg/kg bw per day for skeletal malformations (increased incidence of fetuses and of litters containing fetuses with scoliosis and 27 presacral vertebrae) in a study of developmental toxicity in rabbits, and using a safety factor of 1000 in consideration of the nature of the effects caused. The 2002 JMPR concluded that the 1000-fold safety factor might be excessive, and that the ARfD of dimethipin should be reconsidered on the basis of appropriate data.

Evaluation for an acute reference dose

The present Meeting reconsidered the ARfD for dimethipin. The study of developmental toxicity in rabbits was re-evaluated in the light of a larger set of historical control data for Dutch belted rabbits, provided by the sponsor (Schroeder, 1999).

1. Toxicological studies: developmental toxicity

Rabbits

Groups of 16 sexually mature female Dutch belted rabbits were artificially inseminated and were intubated with technical-grade dimethipin (purity, 98.3%) at a dose of 0, 7.5, 20, or 40 mg/kg bw per day as a suspension in 0.5% carboxymethyl cellulose at a constant volume of 1 ml/kg bw, on days 6 to 27 of gestation, the day of insemination being considered as day 0 of gestation. The rabbits were killed on day 28 of gestation, and the uterine contents were examined. All fetuses, including those that were aborted or dead, were examined grossly and for skeletal and visceral abnormalities. Statements of adherence to good laboratory practice (GLP) and quality assurance (QA) were provided.

The historical control data in the study report came from an unspecified number of studies carried out over an unspecified period in Dutch belted rabbits. For the re-evaluation, the sponsor provided a larger set of historical control data on the incidences of malformations

Table 1. Fetal and litter incidences of scoliosis and 27 presacral vertebrae

Finding	Present study[a]				Historical control data in study report			Historical control data provided by sponsor		
	Dose (mg/kg bw per day)				Mean overall incidence	Range	N	Mean overall incidence	Range	N
	0	7.5	20	40						
Scoliosis										
Fetal incidence (%)	0	0	0	4.0	0.7	0–1.9	951	0.9	0–4.1	2908
Litter incidence (%)	0	0	0	23.1	4.7	0–12.5	149	4.6	0–20	461
27 presacral vertebrae										
Fetal incidence (%)	7.0	4.8	16.9	16.0	7.2	1.8–14.1	951	7.1	0–23.3	2908
Litter incidence (%)	25.0	14.3	28.6	46.2	24.2	13.3–44.4	149	25.4	0–63.6	461

[a] The number of fetuses examined in the groups receiving dimethipin at a dose of 0, 7.5, 20 and 40 mg/kg bw per day were respectively 71, 84, 77 and 75. The number of litters examined in the groups receiving dimethipin at a dose of 0, 7.5, 20 and 40 mg/kg bw per day were respectively 12, 14, 14 and 13

observed in Dutch belted rabbits in studies performed at the same laboratory during 1977–1983.

No deaths occurred in the does. A slightly increased number of females at 20 and 40 mg/kg bw per day had a reduced amount of faeces beneath the cage at various intervals during gestation when compared with concurrent controls. No data on food consumption were available. Does at 40 mg/kg bw per day showed weight loss between days 6 and 12 of gestation. In this group maternal weight gain between day 6 and 28 was virtually absent. Slight reductions in body weight gain were observed in the middle and low dose group between days 6 and 28. The fertility rate was 88–94% in control and treated groups. At each dose at 0, 20, and 40 mg/kg bw per day one doe aborted on day 28; seven non-viable fetuses were found in does at 0 and 20 mg/kg bw per day (but not at 7.5 and 40 mg/kg bw per day); and three late resorptions occurred in the doe at 40 mg/kg bw per day. At terminal sacrifice, the gross pathological findings in treated does were comparable to those in the controls. No significant differences were found between controls and treated groups in the mean numbers of corpora lutea, implantations, or viable or non-viable fetuses, or in fetal weight. The sex ratio of fetuses at 40 mg/kg bw per day was altered, the mean number of females being reduced. There was no apparent dose- or compound-related increase in the frequency of fetal soft-tissue abnormalities. At 40 mg/kg bw per day, the incidence of scoliosis and 27 presacral vertebrae was increased in fetuses as well as in litters (Table 1). At 20 mg/kg bw per day, an increased incidence in 27 presacral vertebrae was observed in fetuses.

The Meeting noted that the mean overall incidences of scoliosis and 27 presacral vertebrae according to the historical control data in the study report were similar to those in the historical control data for 1977–1983. The ranges for the historical control data for 1977–1983 are larger than those in the historical control data in the study report, which reflects the larger number of animals and litters in this database.

In the group receiving dimethipin at a dose of 40 mg/kg bw per day, the fetal and litter incidences of 27 presacral vertebrae exceeded the upper ranges of the historical control data in the study report. However, ranges were were within those for the historical control data for 1977–1983. The increase in fetal incidence of 27 presacral vertebrae at 20 mg/kg bw per day was also within the range for the historical control data for 1977–1983.

For scoliosis, the fetal and litter incidences in the group receiving dimethipin at a dose of 40 mg/kg bw per day were respectively similar to and exceeded the upper ranges of the historical control data for 1977–1983. Therefore, the Meeting concluded that the increased incidences of fetuses and litters containing fetuses with scoliosis in the group at 40 mg/kg bw per day were toxicologically relevant.

The NOAEL for maternal toxicity was 20 mg/kg bw per day on the basis of reductions in body weight between days 6 and 12 of gestation, and the absence of body-weight gain during days 6 and 28 of gestation. The NOAEL for developmental toxicity was 20 mg/kg bw per day on the basis of the increased incidence in skeletal malformations (scoliosis) at 40 mg/kg bw per day (McMeekin et al., 1981).

Comments

In a study of developmental toxicity in rabbits, does treated with dimethipin at a dose of 40 mg/kg bw per day showed body-weight loss on days 6 to 12 of gestation and decreased body-weight gain on days 6 to 28 of gestation. Fetal and litter incidences of scoliosis were 0% and 0% in the controls, and 4.0% and 23.1% at 40 mg/kg bw per day, respectively. The observed incidence of scoliosis at 40 mg/kg bw per day was at the upper bound of that for historical controls (i.e. fetal and litter incidences of 4.1% and 20%, respectively). The NOAEL for both maternal and developmental toxicity was 20 mg/kg bw per day.

Toxicological evaluation

The Meeting established an ARfD of 0.2 mg/kg bw based on the NOAEL of 20 mg/kg bw per day in the study of developmental toxicity in rabbits and a safety factor of 100. The Meeting considered that a safety factor of 100 was adequate, since the observed developmental toxicity was at the upper range of the historical control incidence, and was possibly secondary to maternal toxicity.

Estimate of acute reference dose

0.2 mg/kg bw

References

McMeekin, S.O., Schardein, J.L. & Blair, M. (1981) N252 (Harvade®technical). Teratology study in rabbits. Unpublished report from International Research and Development Corporation, Inc., USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.

Schroeder, R.E. (1999) Audited tabulation of IRDC developmental toxicity historical control data for Dutch belted rabbits (1997–1983). Unpublished report from MPI Research. USA. Submitted to WHO by Uniroyal Chemical Co., Inc., Bethany, Connecticut, USA.

FENPROPIMORPH (addendum)

First draft prepared by
Rudolf Pfeil
Federal Institute for Risk Assessment, Berlin, Germany

Explanation

Fenpropimorph is a morpholine fungicide with systemic activity, interfering with sterol biosynthesis. It was first evaluated by the 1994 JMPR, which established an acceptable daily intake (ADI) of 0–0.003 mg/kg bw on the basis of a no-observed-adverse-effect level (NOAEL) of 10 mg/kg of diet, equal to 0.3 mg/kg bw per day, in a 2-year study of toxicity and carcinogenicity in rats. At the 2001 JMPR, an acute reference dose (ARfD) of 1 mg/kg bw was established on the basis of a NOAEL of 100 mg/kg bw per day in a study of acute neurotoxicity in rats. In 2002, the government of the Federal Republic of Germany asked the Meeting to reconsider the ARfD established for fenpropimorph by the 2001 JMPR, because this government considered that the NOAEL of 15 mg/kg bw per day for teratogenicity in the rabbit to be a more appropriate basis for the ARfD. The present review was undertaken to determine the appropriate end-point and NOAEL for establishing an ARfD, and to evaluate a new screening study of pre- and postnatal developmental toxicity in rats, which was submitted to the present Meeting.

Evaluation for acute reference dose

Rats

In a screening study of prenatal/postnatal developmental toxicity, which complied with with the principles of good laboratory practice (GLP), groups of 10 mated female Wistar rats (Crl:WI(GLX/BRL/HAN)IGS BR) received diets containing fenpropimorph (purity, 96.5%) at a dose of 0, 5, 10 or 15 mg/kg bw per day during pregnancy and lactation (up to day 21 post partum). The study was performed roughly according to the test guidelines of the United States Environmental Protection Agency (OPPTS 870.3800) and the OECD (TG 415), with the major deviation that only females were treated. The target doses were adjusted regularly throughout the study; the actual mean intakes of fenpropimorh were 0, 5.4, 10.3 and 15.5 mg/kg bw per day during gestation and 0, 5.0, 9.5 and 13.3 mg/kg bw per day during lactation. The F_0 females were allowed to litter and rear their F_1 pups until day 4 (reduction of litter size) or day 21 after parturition. After the F_1 pups were weaned, the F_0 dams were sacrificed. The state of health of the F_0 dams and pups was checked each day. Food consumption of the F_0 dams was determined regularly during pregnancy (days 0, 7, 14 and 20) and lactation (days 1, 4, 7 and 14). Body weights of the F_0 dams were determined regularly during pregnancy (days 0, 7, 14 and 20) and lactation (days 1, 4, 7, 14 and

21). The F_1 pups were weighted on the day after birth and on day 4, 7, 14 and 21 post partum, and their viability was recorded. Before sacrifice, blood samples were taken from the fasted F_0 dams for assessment of standard haematological and biochemical parameters, including cholinesterase activity. All F_0 dams were examined for gross pathology (including weights of brain and liver) and brain tissue was sampled and examined for cholinesterase activity.

In F_0 females, food consumption was decreased during pregnancy (94%, 88%, and 85% of controls) and lactation (79, 74, and 66% of controls) at all doses. Body-weight gain was reduced transiently during the first week of gestation at the lowest dose and throughout gestation at the intermediate and highest doses. Statistically significantly decreased serum cholinesterase activity was observed at all doses. No inhibition of erythrocyte or brain cholinesterase activities and no treatment-related changes in other clinical chemistry or haematological parameters were observed. There were no substance-related adverse effect on any parameter involving organ weight or gross pathological findings. There were no adverse effects on reproductive performance.

At birth, mean body weights of pups were reduced in males and females at the highest dose (86% and 92% of that of controls, respectively). During the second and third week of lactation, body weights of the pups were slightly reduced in all groups treated with fenpropimorph when compared with those of the controls. Pup viability was not affected at any dose (Table 1).

Table 1. Selected findings from a study screening for prenatal/postnatal developmental toxicity in rats given diets containing fenpropimorph

	Dose (mg/kg bw per day)			
	0	5	10	15
Parents				
Mated females in study/non-pregnant	10/1	10/0	10/0	10/1
Conception rate (%)	90	100	100	90
Dams with live litters	9	10	10	9
Food consumption during gestation (g/animal per day):				
Days 0–7	18.6	16.8*	15.6**	14.5**
Days 7–14	20.2	19.1	18.0**	17.6**
Days 14–20	22.5	21.5	20.6*	20.1**
Body-weight gain during gestation (g):				
Days 0–7	26.7	21.5	16.8**	14.4**
Days 7–14	26.5	26.2	23.2	23.8
Days 0–20	99.4	93.6	80.4**	76.1**
Offspring				
Pups delivered/mean litter size	86/9.6	92/9.2	78/7.8	71/7.9
Pups surviving days 0–4/viability index	85/100	90/99	78/100	71/100
Pups surviving days 4–21/lactation index	66/100	79/100	70/100	59/100
Pup weight, males/females (g):				
At birth	6.9/6.3	6.3/6.0	6.3/6.3	5.9*/5.8
Day 4, pre-culling	10.3/9.7	9.4/9.1	9.5/9.4	8.6*/8.5
Day 7	15.8/14.7	14.2/13.7	13.9/13.5	12.8**/12.4*
Day 14	30.5/28.8	26.7*/25.9	27.1*/26.2	25.1**/24.7*
Day 21	49.2/46.4	44.1*/42.5	45.0/42.5	41.7**/40.5*
Pup weight gain, males/females (g)				
Days 1–4	3.5/3.4	3.2/3.1	3.2/3.2	2.7*/2.6
Days 4–7	5.4/5.0	4.8/4.6	4.4*/4.0	4.3*/3.9*
Days 7–14	14.8/14.1	12.5/12.2	13.2/12.7	12.3/12.3
Days 14–21	18.7/17.5	17.4/16.6	17.9/16.3	16.5/15.8
Days 4–21	38.9/36.7	34.6/33.4	35.4/33.0	33.1*/32.0

From Schilling et al. (2000)

* $p < 0.05$, ** $p < 0.01$; Dunnet-test (two-sided); *** $p < 0.002$; Mann-Whitney U-test (two-sided)

The NOAEL for maternal toxicity was <5 mg/kg bw per day on the basis of decreased food consumption during pregnancy and lactation and reduced body-weight gain during pregnancy, at all doses. The NOAEL for developmental toxicity was <5 mg/kg bw per day on the basis of reduced body weights/body-weight gains of pups at all doses during lactation (Schilling et al., 2000).

In a study of prenatal developmental toxicity, conducted according to the test guidelines of the United States Food and Drug Administration and the Association of the British Pharmaceutical Industry, groups of 26–31 pregnant Sprague-Dawley rats were given fenpropimorph (purity, 92.5%; dissolved in olive oil) at a dose of 0, 2.5, 10, 40 or 160 mg/kg bw per day by gavage on days 6–15 of gestation. Dams were observed for clinical symptoms, body weight, mortality, macroscopic appearance of internal organs, conception rate and numbers of corpora lutea, viable implantations and dead implantations (early, intermediate and late resorptions). Fetuses were examined for weight, length, placental weight and external, skeletal and visceral signs.

Vaginal bleeding was observed in one animal in the control group, one animal at 10 mg/kg bw per day, three animals at 40 mg/kg bw per day and 16 animals at 160 mg/kg bw per day. A dose-related reduction in body-weight gain was observed in dams at doses of ≥40 mg/kg bw per day during the treatment period and at 160 mg/kg bw per day during the whole observation period, starting from day 6. At 160 mg/kg bw per day, the body weights of the dams were reduced and the number of viable fetuses was decreased, in association with an increased number of dead implants. The weight and length of the fetuses were reduced, and placental weight was increased. Irreversible structural changes, including cleft palate (fourteen fetuses from seven litters) and inferior brachygnathia (one fetus), were observed at the highest dose (Table 2).

Table 2. Selected findings from a study of prenatal developmental toxicity in rats given fenpropimorph by gavage

	Dose (mg/kg bw per day)				
	0	2.5	10	40	160
Pregnant females in study	26	31	30	29	31
Vaginal bleeding	1	0	1	3	16
Body-weight gain, days 6–15 (g)	36	33	32	25**	–21**
Completely resorbed litters	0	0	0	0	7**
Mean No. of corpora lutea	13.5	14.1	14.3	14.3	13.5
Mean No. of implantations	10.7	9.7	10.7	11.8	10.7
Mean No. of viable fetuses	9.9	9.0	10.2	11.2	8.8
Mean No. of dead implantations	0.85	0.65	0.50	0.66	1.87**
No. of early resorptions (Salewski method)	16 (0)	16 (0)	9 (0)	8 (0)	9 (39)
No of intermediate/late resorptions	6/0	3/1	5/0	6/3	8/2
No. of dead fetuses	0	0	1	2	0
Sex ratio (% males/litter)	51	54	54	54	53
Litter weight (g)	26	31	30	29	24
Fetal weight, males/females (g)	3.50/3.42	3.66/3.42	3.80/3.56	3.77/3.56	3.30/3.14**
Fetal length, males/females (mm)	3.55/3.50	3.59/3.49	3.63/3.53	3.57/3.53	3.43**/3.36**
Placental weights	0.61	0.65	0.61	0.64	0.75**
Malformations, No. (%) of fetuses	12 (4.4)	7 (2.6)	8 (2.9)	12 (3.2)	19 (7.4)
Malformations, No. (%) of litters	6 (23.1)	7 (22.6)	6 (20.0)	6 (20.7)	11 (45.8)
Cleft palate, No. of fetuses/litters	0	0	0	0	14/7
Brachygnathia, No. of fetuses/litters	0	0	0	0	1/1
Variations/retardations, No. (%) of fetuses	42 (15.0)	46 (17.1)	21 (8.6)	40 (11.2)	36 (14.9)
Variations/retardations, No. (%) of litters	16 (61.5)	17 (54.8)	11 (36.7)	16 (66.7)	16 (66.7)

From Hofmann & Merkle (1978)
** $p < 0.01$

The NOAEL for maternal toxicity was 10 mg/kg bw per day, on the basis of clinical signs (vaginal bleeding) and reduced body-weight gain in dams at 40 mg/kg bw per day and above. The NOAEL for developmental toxicity was 40 mg/kg bw per day, on the basis of evidence for embryo-/fetotoxicity and teratogenicity at 160 mg/kg bw per day (Hofmann & Merkle, 1978).

Rabbits

In a study of prenatal developmental toxicity, which was conducted according to the test guidelines of the United States Environmental Protection Agency, groups of 15 female Himalayan Chbb:HM rabbits were given fenpropimorph (purity, 92.5%) at a dose of 0, 2.4, 12 or 60 mg/kg bw per day by gavage in 0.5% carboxymethylcellulose (5 ml) during days 6–18 of gestation (experiment I). As dams at the highest dose showed severe signs of toxicity, an additional group of 15 animals treated with fenpropimorph at 36 mg/kg bw per day, and a second control group of 10 animals were included in the study (experiment II). All surviving animals were sacrificed on day 29 post insemination and the fetuses were delivered by caesarean section.

Diarrhoea was seen in all groups, and increased in incidence and severity with the dose. At 60 mg/kg bw per day, severe diarrhoea, salivation, apathy, a greenish mucous discharge from the nose and encrustations in the vaginal region and snout were seen, and convulsions were observed before the deaths of 11 animals. Food consumption and body weight were reduced. Macroscopic examination of the animals that died at this dose showed dilatation of the right chamber of the heart and congestive hyperaemia; the clinical findings were confirmed.

No dose-related effects on the number of corpora lutea, pre-implantation loss or conception rate were observed in dams, and no effects were seen on visceral examination of the fetuses.

At 60 mg/kg bw per day, absolute weights of the uterus were reduced, and the numbers of early resorptions and dead fetuses were increased such that only one fetus survived. This fetus had several abnormalities, including syndactyly on the forelegs, an anomalous position of the hindlegs and micromelia, and reduced weight and length; furthermore, the placental weight was increased, and the individual sternebrae were fused.

At 36 mg/kg bw per day, the dams had clinical signs that were less severe and occurred at a lower incidence than those in the group receiving the highest dose. Two animals aborted and three were killed in extremis. The numbers of dead implantations (attributable mainly to early resorptions) were slightly increased at this dose. Six fetuses from two litters had pseudoankylosis, a skeletal variation, which was also found in one fetus at 12 mg/kg bw per day (Table 3).

The NOAEL for maternal toxicity was 12 mg/kg bw per day on the basis of clinical sings (diarrhea, salivation) and reduced body-weight gain at ≥36 mg/kg bw per day. The NOAEL for developmental toxicity was 12 mg/kg bw per day, on the basis of evidence for embryo- and fetotoxicity at ≥36 mg/kg bw per day (Zeller & Merkle, 1980).

In a study of prenatal developmental toxicity, which was conducted in compliance with the principles of GLP and according to the test guidelines of the United States

Table 3. Selected findings from a study of prenatal developmental toxicity in rabbits given fenpropimorph by gavage

	Dose (mg/kg bw per day)					
	Experiment I				Experiment II	
	0	2.4	12	60	0	36
No. of animals inseminated/pregnant	15/14	15/14	14/11	15/14	10/8	15/13
Conception rate	93%	93%	79%	86%	80%	93%
Mortalities/sacrificed prematurely	0/5	1/5	0/3	11/0	0/1	0/5
Abortions/premature birth	0/5	0/5	0/3	0/0	0/1	2/0
Clinical signs in dams[b]	+/–	+/–	+	+++	+/–	++
Food intake, days 7–12 (g/day)	71	70	68	42**	69	57
Food intake, days 13–19 (g/day)	77	73	75	6**	47	46
Body-weight change, days 7–19 (g)	+78	+6	+106	–542**	–6	–45
No. of completely resorbed litters/live litters	0/9	0/8	0/8	3/1	0/7	0/9
Gravid uterus weight (g)	326	444	468	27**	333	284
Mean No. of corpora lutea/implantations	9.9/6.4	7.5/6.9	9.6/8.1	8.3/5.8	10.7/7.1	9.9/8.0
Mean No. of live fetuses	6.2	6.5	7.4	1**	6.4	5.9
Pre-implantation loss (%)	33	10	13	14	32	30
No. of live litters with a postimplantation loss of >2	0	0	0	1 (100%)	0	3 (33%)
Placental weight (g)	4.6	4.5	4.1	5.1	4.6	4.3
Fetal weight (g)	38.8	38.3	37.0	37.4	38.4	35.8
Crown–rump length (cm)	8.4	8.4	8.2	8.1	8.1	7.8
Malformations (% fetuses)	0	0	5.4	[a]	5.9	10.0
Malformations (% litters)	0	0	37.5	[a]	42.9	33.3
Variations/retardations (% fetuses)	73.4	79.3	68.5	[a]	89.4	86.5
Variations/retardations (% litters)	100	100	87.5	[a]	100	100
Pseudoankylosis, No. of fetuses	0	0	1	[a]	0	6

From Zeller & Merkle (1980)
** $p < 0.01$
[a] Only one fetus survived
[b] + = yes, – = no

Environmental Protection Agency and the OECD (TG 414), groups of 20 pregnant Russian Chbb:HM rabbits were given fenpropimorph (purity, 95.6%; in 0.5% aqueous sodium carboxymethylcellulose) at a dose of 0, 7.5, 15 or 30 mg/kg bw per day by gavage on days 7–19 of gestation.

There were no mortalities in the dams throughout the study. The only clinical sign related to treatment was swelling of the anus for 2 to 12 days, observed in 9 rabbits at the highest dose. Also at this dose, food consumption was reduced during the whole treatment period, while maternal body weight was slightly reduced from days 12 to 29, body-weight gain was significantly reduced during days 7 to 15, and four animals showed weight loss. No maternal toxicity was observed at the lower doses.

There were no effects on pre- or postimplantation loss, number of live or dead fetuses per litter or sex ratio. Mean gravid uterus weight and weights of male fetuses were significantly reduced at the highest dose. At the highest dose of 30 mg/kg bw per day, there was an increase in the total number of malformations (21 fetuses from 4 litters) and findings described as 'anomalies' (36 fetuses from 13 litters). The malformations occurred mainly in the litters of three dams that showed marked signs of toxicity during treatment. Twenty fetuses from three litters had shortened fore- and hindlimbs, and four fetuses from two litters had a cleft palate. One of these fetuses also had exencephaly and open eye, and another fetus had gastro- and cranioschisis, an asymmetric skull and oedema of the trunk. One fetus in another litter had a diaphragmatic hernia. Furthermore, abnormal positions of forelimbs were observed in twenty-five fetuses from seven litters and of the hindlimbs in

Table 4. Selected findings from a study of prenatal developmental toxicity in rabbits given fenpropimorph by gavage

	Dose (mg/kg bw per day)			
	0	7.5	15	30
No. of females on study/pregnant at caesarean section	20/18	20/20	20/20	20/20
Body-weight gain over whole treatment period (g)	140	96	103	42**
Mean No. of corpora lutea/implantations	8.8/7.2	8.8/7.1	8.8/7.5	7.8/6.3
Mean No. of early/late resorptions	0.2/0	0.3/0.1	0.5/0.1	0.4/0.1
Mean pre-/postimplantation loss (%)	17.7/0.2	18.7/0.4	14.4/0.6	19.6/0.5
Total/mean No. of live fetuses	126/7.0	134/6.7	138/6.9	116/5.8
Mean sex ratio (% males)	54	43	52	47
Mean gravid uterus weight (g)	369	347	356	287*
Mean fetal weight, males/females (g)	38.0/38.0	37.6/37.4	38.0/35.2	34.7*/35.1
Shortened fore- and hindlimbs (No. of fetuses/litters)	0	0	0	20/3[a]
Cleft palate (No. of fetuses/litters)	0	0	0	4/2
Abnormal positions of forelimbs (No. of fetuses/litters)	0	0	0	25/7[b]
Abnormal positions of hindlimbs (No. of fetuses/litters)	0	0	0	8/3[b]

From Marty (1993)
*$p < 0.05$, **$p > 0.01$
[a] Finding not reported in historical controls
[b] Historical controls: 0–3.7% of fetuses, 0–20% of litters

eight fetuses from three litters (Table 4). The external findings were confirmed by skeletal examination.

The NOAEL for maternal toxicity was 15 mg/kg bw per day on the basis of clinical signs (swelling of the anus) and reduction of food consumption and body-weight gain at 30 mg/kg bw per day. The NOAEL for developmental toxicity was 15 mg/kg bw per day on the basis of evidence for embryo- and fetotoxicity and teratogenicity at 30 mg/kg bw per day (Marty, 1993).

Comments

Fenpropimorph is of low acute toxicity; in rats, the oral median lethal dose (LD_{50}) was 1500–3500 mg/kg bw, the dermal LD_{50} was 4300 mg/kg bw, and the inhalation median lethal concentration (LC_{50}) was 2.9 mg/l of air (studies evaluated by the JMPR in 1994 and 2001; Annex 1, references 73 and 94.

In a study of acute neurotoxicity in rats, the NOAEL was 100 mg/kg bw per day on the basis of clinical and behavioural signs observed at doses of 500 and 1500 mg/kg bw per day.

In a screening study of pre- and postnatal developmental toxicity in rats, the NOAEL for maternal toxicity was <5 mg/kg bw per day on the basis of decreased food consumption during pregnancy and lactation, and reduced body-weight gain during pregnancy, at all doses. The NOAEL for developmental toxicity was <5 mg/kg bw per day on the basis of reduced body weight or body-weight gain in pups at all doses during lactation.

In a study of prenatal developmental toxicity in rats, an increased incidence of cleft palate (fourteen fetuses from seven litters) was observed at the highest dose of 160 mg/kg bw per day. At this dose, severe maternal toxicity, including mortality, was found. The

NOAEL for developmental toxicity was 40 mg/kg bw per day, while the NOAEL for maternal toxicity was 10 mg/kg bw per day.

In a study of prenatal developmental toxicity in Himalayan rabbits, severe maternal toxicity, including mortality, was found at 60 mg/kg bw per day, the highest dose tested. The number of early resorptions and dead fetuses was increased such that only one fetus survived. This fetus had several abnormalities, including syndactyly of the forelimbs, an anomalous position of the hindlimbs and micromelia, fusion of individual sternebrae, and reduced weight and length. At 36 mg/kg bw per day, the clinical signs in dams (diarrhoea, salivation) were less severe and occurred at a lower incidence than at the highest dose. Two animals aborted and three were killed in extremis. The number of postimplantation losses was slightly increased at this dose. Six fetuses from two litters had pseudoankylosis, a skeletal variation. The NOAEL was 12 mg/kg bw per day for both maternal toxicity and developmental toxicity.

In a study of prenatal developmental toxicity in Russian rabbits, maternal toxicity (swelling of the anus, reduction of food consumption and of body weight or body-weight gain, weight loss) was observed only at the highest dose tested, 30 mg/kg bw per day. There were no effects on pre- or postimplantation loss, number of live or dead fetuses per litter and sex ratio. Mean gravid uterus weight and weights of male fetuses were significantly reduced at the highest dose. There was an increase in the total number of malformations (21 fetuses from four litters) and in findings described as "anomalies" (36 fetuses from 13 litters). The malformations occurred mainly in the litters of three dams that showed marked signs of toxicity during treatment. Twenty fetuses from three litters had shortened fore- and hindlimbs, and four fetuses from two litters had a cleft palate. Furthermore, position anomalies were observed in the forelimbs in twenty-five fetuses from seven litters and in the hindlimbs in eight fetuses from three litters. The NOAEL was 15 mg/kg bw per day for both maternal toxicity and developmental toxicity.

Toxicological evaluation

The Meeting established an ARfD of 0.2 mg/kg bw on the basis of an overall NOAEL of 15 mg/kg bw per day for embryo- and fetotoxicity and teratogenicity in two studies of prenatal developmental toxicity in rabbits, and using a safety factor of 100. The lowest-observed-adverse-effect level (LOAEL) of 5 mg/kg bw per day for decreased body-weight gain in pups in the screening study of pre- and postnatal developmental toxicity in rats is related to repeated pre- and postnatal exposure, and is therefore not considered to be an appropriate basis for establishing an ARfD.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	Acute neurotoxicity	Neurotoxicity	100 mg/kg bw per day	500 mg/kg bw per day
	Screening for prenatal and postnatal developmental toxicity	Maternal toxicity	<5 mg/kg bw per day	5 mg/kg bw per day
		Developmental toxicity	<5 mg/kg bw per day[a]	5 mg/kg bw per day[a]
	Prenatal developmental toxicity	Maternal toxicity	10 mg/kg bw per day	40 mg/kg bw per day
		Developmental toxicity	40 mg/kg bw per day	160 mg/kg bw per day
Rabbit	Prenatal developmental toxicity	Maternal toxicity	12 mg/kg bw per day	36 mg/kg bw per day
		Developmental toxicity	12 mg/kg bw per day	36 mg/kg bw per day
	Prenatal developmental toxicity	Maternal toxicity	15 mg/kg bw per day	30 mg/kg bw per day
		Developmental toxicity	15 mg/kg bw per day	30 mg/kg bw per day

[a] NOAEL and LOAEL for an effect that is not considered relevant for a single exposure

Estimate of acute reference dose

0.2 mg/kg bw

References

Hofmann, H.T. & Merkle, J. (1978) Investigation to determine the prenatal toxicity of 4-[3-[4-(1,1-dimethyl ethyl)phenyl]-2-methyl]propyl-2,6(*cis*)-dimethyl morpholine on rats. Unpublished report No. 78/043, dated 6 October 1978, from BASF AG, Ludwigshafen, Germany. Submitted to WHO by BASF AG, Ludwigshafen, Germany.

Marty, J.H. (1993) CGA 101031 technical: rabbit oral teratogenicity. Unpublished report No. 93/11016, study No. 923154, dated 29 June 1993, from Ciba-Geigy Ltd, Toxicology Services, Stein, Switzerland. Submitted to WHO by BASF AG, Ludwigshafen, Germany.

Schilling, K., Deckardt, K., Gembardt, C. & Hildebrand, B. (2000) Fenpropimorph—pre-/postnatal screening toxicity study in Wistar rats. Continuous dietary administration. Unpublished report No. 2000/101325, project No. 62R0047/93068, dated 4 August 2000, from BASF AG, Ludwigshafen, Germany. Submitted to WHO by BASF AG, Ludwigshafen, Germany.

Zeller, H. & Merkle, J. (1980) Study to determine the prenatal toxicity of 4-[3-[4-(1,1-dimethylethyl)phenyl]-2-methyl]propyl-2,6(*cis*)-dimethyl morpholine (regulatory No. 108 406) in rabbits. Unpublished report No. 80/0109, dated 22 April 1980, from BASF AG, Ludwigshafen, Germany. Submitted to WHO by BASF AG, Ludwigshafen, Germany.

FENPYROXIMATE (addendum)

First draft prepared by
T.C. Marrs
Food Standards Agency, London, England

Explanation

Evaluation for an acute reference dose

Fenpyroximate is a phenoxypyrazole acaricide. Fenpyroximate was evaluated by the 1995 JMPR, when an acceptable daily intake (ADI) of 0–0.01 mg/kg bw was established based upon a no-observed-adverse-effect level (NOAEL) of 1 mg/kg bw per day in a 104-week study in rats and a safety factor of 100. The critical effect in that study was a reduction in body-weight gain. Fenpyroximate was re-evaluated by the present Meeting in order to determine an acute reference dose (ARfD).

1. Toxicological studies

1.1 Short-term studies of toxicity

Mice

A 4-week range-finding study in SPF ICR (Crj:CD-1) mice was undertaken. This was to determine the doses suitable for use in an 18-month study of carcinogenicity. Groups of nine males and nine females (aged 4 weeks) were given diets containing fenpyroximate (purity, 97.9%) at a concentration of 0, 20, 100 or 500 ppm, producing intakes of test material of 0, 2.58, 12.9 and 53.2 mg/kg bw per day for males and 0, 3.07, 14.5 and 66.7 mg/kg bw per day for females. The animals were observed daily. Food consumption was measured weekly. After 4 weeks, urine analysis was carried out. Blood was taken for haematological and clinical chemistry examination (including cholinesterase activity) after 4 weeks of treatment. At autopsy, the weights of selected organs were recorded. Pathological examination by microscopy was not carried out. No abnormal clinical signs were observed, and no deaths occurred. There was a decrease in body weight compared with that of the controls at weeks 1, 2 and 4 in the males and at week 4 in the females at 500 ppm. In the same group, food consumption was depressed in the males at weeks 1 and 4 and at week 1 at 100 ppm. The specific gravity of the urine was reduced in male mice at 100 and 500 ppm. Notable changes in haematological parameters were not seen. A decrease in

alanine aminotransferase activity was seen in male mice and an increase in blood urea nitrogen in female mice, both at the highest dietary concentration only. No test-material-related changes were noted in plasma or erythrocyte cholinesterase activities. No test-material related changes in gross pathology or organ weights were observed. The NOAEL for the study was 20 ppm (equal to 2.58 and 3.07 mg/kg bw per day in males and females respectively) on the basis of reduced food consumption at week 1 in males at 100 ppm (Takahashi, 1987).

Another 4-week range-finding study in SPF ICR (Crj:CD-1) mice was undertaken to determine the doses suitable for use in an 18-month study of carcinogenicity. A second range finding study was considered necessary, as few changes were found in the first (see above; Takahashi, 1987). Groups of nine males and nine females (aged 5 weeks) were given diets containing fenpyroximate (purity, 97.9%) at a concentration of 0, 80, 400 or 2000 ppm. These diets produced intakes of fenpyroximate of 0, 10.8, 48.4 and 182 mg/kg bw per day for males and 0, 11.7, 50.4 and 170 mg/kg bw per day for females. The animals were observed daily and the numbers of animals killed in extremis or found dead were recorded. Body weights were recorded weekly and food consumption was recorded over each period of 7 days. Urine analysis was performed on survivors at 4 weeks. Blood was taken for haematological and clinical chemistry examination (including cholinesterase activity) after 4 weeks of treatment. At autopsy, the weights of selected organs were recorded. Pathological examination by microscopy was not carried out. No deaths were seen and no clinical sign attributable to fenpyroximate was observed. At 2000 ppm, there was decreased body-weight gain and food consumption, while at 400 ppm in males there was decreased body-weight gain. Haematological examination showed decreases in erythrocyte volume fraction, haemoglobin concentration and total leukocyte count in males, and in haemoglobin concentration, erythrocyte count and mean corpuscular haemoglobin in females. An increase in aspartate aminotransferase activity was seen in both sexes and a decrease in total plasma protein was seen, also in both sexes. At 2000 ppm in males, absolute weights of the brain, pituitary, heart, thymus, kidney and testis were decreased. There was also, at this dietary concentration in the males, increases in the relative weights of the brain, thyroid, heart and adrenals and decreases in relative weights of the thymus and spleen. In the females at the highest dietary concentration, there were decreases in the absolute weight of the brain, pituitary, thyroid, heart, thymus, liver, kidney, spleen, adrenal and ovaries and an increase in the relative weight of the brain, heart and liver, and a decrease in the relative weight of the spleen and ovaries. At 400 ppm, the only finding of interest was a decrease in body-weight gain in males; on the basis of this finding, the NOAEL was 80 ppm, equal to 10.8 mg/kg bw per day for males and 11.7 mg/kg bw per day for females (Takahashi, 1988).

Rats

A 13-week study in CD rats was undertaken to assist in determining the doses for a 2-year combined study of carcinogenicity and toxicity. Groups of 10 males and 10 females were given diets containing fenpyroximate (purity, 99.0%) at a concentration of 0, 20, 100 or 500 ppm (equal to 0, 1.30, 6.57 and 35.2 mg/kg bw per day for males and 0, 1.65, 8.29 and 38.6 mg/kg bw per day for females. The rats were examined twice daily and any clinical signs recorded. A more detailed physical examination was carried out weekly. Body weights were estimated weekly, as was food consumption. Ophthalmoscopy was carried out before the start of the experiment on all rats and at 13 weeks in the controls and in the rats fed fenpyroximate at the highest dietary concentration. Blood was taken at 13 weeks for haematological and clinical chemistry examination. Urine analysis was undertaken at 13 weeks. After the rats had been killed, selected organs were removed, weighed and processed

for microscopical examination. A total of three intercurrent mortalities occurred (two males at the highest dietary concentration, and one female at 20 ppm). None of these fatalities was believed to be related to the administration of the test material. No abnormal clinical signs were seen, but hair loss and encrustations/excoriations of the skin were seen in females at 500 ppm; a few males at this dose had encrustations of the muzzle. Body-weight gain in both sexes at 100 and 500 ppm was lower than that in controls (markedly so at 500 ppm). Food consumption was markedly lower in both sexes in rats receiving fenpyroximate at a dietary concentration of 500 ppm. No significant abnormality was found on ophthalmoscopy. At 500 ppm, there was an increase in erythrocyte volume fraction, haemoglobin concentration and erythrocyte count, as well as a decrease in total leukocyte count in males and an increase in erythrocyte volume fraction, haemoglobin concentration and erythrocyte count in females. At 100 ppm, there was a decrease in total leukocyte count in males. Lowered total protein was seen in both sexes at 500 ppm and in females at 100 ppm. Lower plasma butyrylcholinesterase activity was seen in females at 500 ppm. A rise in the activity of alkaline phosphatase was observed in females at 500 ppm. In rats treated with fenpyroximate at 500 ppm for 12 weeks, the volume and pH of the urine were decreased in males and the specific gravity was decreased in females. No treatment-related intergroup differences were seen in organ weights. At autopsy, facial hair loss was noted in females at 500 ppm. At microscopy, minimal hepatocyte hypertrophy was seen in both sexes at 100 ppm and 500 ppm. The NOAEL for the study was 20 ppm, equal to 1.30 mg/kg bw per day for males and 1.65 mg/kg bw per day for females, on the basis of minimal hepatocyte hypertrophy at the higher doses (Aughton, 1987).

Dogs

In a 13-week study, groups of four male and four female dogs received capsules containing fenpyroximate (purity, 98.4–98.6%) at a dose of 2, 10 or 50 mg/kg bw per day. Controls (four dogs of each sex per group) received the empty gelatin capsules. Dogs were inspected throughout the working day and daily observation of each animal was carried out. A more detailed weekly examination was also carried out. A detailed veterinary examination was carried out before the start of the study and after 4, 8 and 12 weeks of treatment. Ophthalmoscopic examination of the eyes was undertaken after 4, 8 and 12 weeks of treatment. Debilitated animals were carefully observed and those in extremis were killed, blood samples having been taken ante mortem. Body weight was measured at the start of the study, thence weekly and before death. Food consumption was measured daily. Water consumption was measured during 3 days in week 6. Electrocardiography was performed before the start of treatment and at weeks 6 and 12; at weeks 6 and 12, electrocardiography was performed both 2 h and 24 h after dosing. Before the start of treatment and after 6 and 12 weeks of dosing, blood was taken for haematological investigations and clinical chemistry studies; during the treatment period, samples were taken before dosing. Urine analysis was carried out before the start of the study and after 11 weeks of treatment. Surviving dogs were killed at 12 weeks and a detailed necropsy undertaken. Selected organs were removed and weighed. Samples of selected organs and any macroscopical abnormalities were processed for histopathological examination. Two females at the highest dose were killed in extremis during the study, because of severe weight loss and loss of appetite. Dogs in all treated groups had diarrhoea, and in the males this appeared to be dose-related and was apparent from week 1 (see Tables 1 and 2).

Emesis was seen in both sexes at 10 and 50 mg/kg bw per day. Emaciation was seen at 50 mg/kg bw per day (and in one female at 2 mg/kg bw per day). Torpor was seen in some females at 2 and 10 mg/kg bw per day, and in males and females at 50 mg/kg bw per

Table 1. The mean percentage[a] of dogs having diarrhoea after treatment with capsules containing fenpyroximate in a 13-week study

Sex	Dose (mg/kg bw per day)			
	0	2	10	50
Males	8.5	22.7	21.2	70.0
Females	5.0	30.8	50.0	48.0

From Broadmeadow (1989)

[a] The percentage of dogs having diarrhoea was recorded each day. The mean percentage of dogs having diarrhoea was calculated by adding the daily percentage for each group and dividing by the number of days on which observations had been carried out

Table 2. Data from individual animals[a] on diarrhoea observed before dosing and during week 1 of dosing

Individual	Dose (mg/kg bw per day)							
	Males				Females			
	0	2	10	50	0	2	10	50
1	0 (3)	0 (4)	0 (4)	0 (7)	0 (0)	0 (5)	0 (5)	0 (3)
2	0 (0)	0 (2)	0 (6)	0 (7)	0 (0)	1 (6)	0 (6)	1 (7)
3	0 (0)	0 (0)	1 (0)	0 (2)	3 (3)	0 (2)	0 (0)	0 (2)
4	0 (0)	0 (1)	0 (1)	0 (6)	0 (0)	0 (0)	0 (1)	0 (0)

From Broadmeadow (1989)

[a] The first figure is the number of days during the week before dosing that each beagle had diarrhoea; the figure in parentheses is the number of days during the first week of dosing that each beagle had diarrhoea

day. Weight loss was seen in week 1, in females receiving fenpyroximate at a dose of 10 mg/kg bw per day and in males and females at a dose of 50 mg/kg bw per day. Body-weight gain was clearly depressed at 50 mg/kg bw per day in males, and at 50 and 10 mg/kg bw per day in females, compared with that of the controls. Body-weight gain in females was marginally depressed, compared with that of the controls, in the group receiving fenpyroximate at 2 mg/kg bw per day. Food consumption was unaffected by treatment in males, but was reduced by treatment in a dose-related fashion in females. No treatment-related ocular lesions were noted. Slight bradycardia was seen in all treatment groups in both sexes, but especially in the groups receiving fenpyroximate at 10 and 50 mg/kg bw per day. There was no consistent difference between the measurements made 2 h after dosing and 24 h after dosing, and the bradycardia was not consistently present at 2 mg/kg bw per day. In males at all doses and in females at 2 and 10 mg/kg bw per day, no differences in haematological parameters were seen, compared with those of the concurrent controls. In females at 50 mg/kg bw per day, low total leukocyte counts at 6 weeks and 12 weeks, prolonged activated partial thromboplastin times at 6 weeks and high platelet counts at 12 weeks were recorded relative to these values for the concurrent controls. The two decedents (both females at 50 mg/kg bw per day) had low leukocyte counts. Raised blood urea nitrogen concentration was seen in females at 50 mg/kg bw per day at week 6, and at 2 and 50 mg/kg bw per day at week 12; it is unclear whether these effects were treatment-related as there was no clear dose–response relationship. Low concentrations of glucose were seen in males at 10 mg/kg bw per day and in both sexes at 50 mg/kg bw per day at weeks 6 and 12. The two decedents (both females at 50 mg/kg bw per day) had high blood urea concentrations and low plasma butyrylcholinesterase activities, and one of them had a low concentration of blood glucose. No intergoup differences were seen in the results of urine analysis. Slightly higher absolute and relative weights of the adrenals were observed in males at 50 mg/kg bw

per day and slightly higher relative weights of the adrenals in females at that dose. Relative weights of the liver were increased in both sexes at 50 mg/kg bw per day. Macroscopic examination post mortem showed emaciation in one surviving female at 50 mg/kg bw per day. The decedents showed emaciation. There was depleted hepatic glycogen and fine renal medullary cytoplasmic vacuolation in the two decedent females at the highest dose, as well as in one surviving female at 50 mg/kg bw per day. The lowest-observed-adverse-effect level (LOAEL) for the study was 2 mg/kg bw per day on the basis of clinical signs at that dose (diarrhoea in both sexes, and torpor in females) and reduced body-weight gain in females. This LOAEL is probably close to the NOAEL (Broadmeadow, 1989).

1.2 Reproductive toxicity

(a) Multigeneration studies

Rats

In a two-generation study of toxicity, groups of male and female CD rats were given diets containing fenpyroximate (purity, 97.3%) at a concentration of 0, 10, 30 or 100 ppm (equal to 0.67, 1.99 and 6.59 mg/kg bw per day for the F_o males and 0.83, 2.44 and 8.60 mg/kg bw per day for the F_o females, and 0.78, 2.33 and 8.45 mg/kg bw per day for the F_1 males and 0.96, 2.82 and 9.92 mg/kg bw per day for the F_1 females). In the F_o generation, there were 24 males and 24 females in each group and they received the test diets for 14 weeks before being paired to produce the F_1 litters; treatment of the F_o parents was continued throughout mating and until weaning of the F_1 offspring, at which time the F_0 males were killed, while the females were killed about 10 days later. At each dietary concentration, the size of these litters was reduced to eight (four males and four females where the numbers of each sex in the litter made this possible). Where the litter size was less than eight, culling was not carried out. Twenty-four males and 24 females were selected as the F_1 generation (if possible, one male and one female being selected from each litter. These animals were given diets containing fenpyroximate, as described above, for 14 weeks, after which they were paired to produce the F_2 litters. Treatment of the F_1 parents via the diet continued throughout mating and until weaning of the F_2 offspring, at which time the F_1 males and the F_2 offspring were killed, while the females were killed about 10 days later. All animals were examined daily throughout the study and any clinical signs were recorded. Animals found dead or killed in extremis were examined macroscopically and abnormal tissues retained. Males were weighed weekly throughout, while females were weighed weekly until mating was detected and thence on days 0, 6, 13 and 20 post coitum and on postnatal days 1, 4, 7, 14, 21 and 25. Food consumption was recorded weekly until pairing. The time between pairing and detection of mating was noted (precoital interval) and the time between detection of mating and commencement of parturition (i.e. the duration of gestation) was recorded. In each generation, on postnatal day 1 the number of offspring borne (alive or dead) was recorded, together with the individual weights and sex. The offspring were examined. Thereafter, daily records were kept of mortality of the offspring. The offspring were weighed on postnatal days 1 and 4 (i.e. before culling) and on postnatal days 7, 14, 21 and 25, and sexed on postnatal days 1 and 4 (before culling) and on postnatal days 14 and 25. Physical development was assessed by recording the time of unfolding of the pinna, growth of body hair, tooth eruption and eye opening. All parental animals were the subject of detailed necropsy and the reproductive organs were weighed (testis, seminal vesicles, prostate and epididymis in males, uterus and ovaries in females) and retained. In the females, the number of uterine implantation sites was recorded. Histopathological examination was carried out on the reproductive organs (testis, seminal vesicles, prostate and

epididymis in males, ovaries, uterus, vagina in females and pituitary in both sexes) and also on any tissue noted at macroscopic examination to be abnormal. Additionally, in those females with litter loss, the mammary glands were examined histopathologically. Offspring found dead, culled at postnatal day 4, F_1 weanlings not selected for continuation of the study (that were killed at age 29–32 days) and F_2 weanlings (that were killed at age 25–27 days) were examined externally and internally for macroscopic abnormalities. In the offspring, any tissue noted at macroscopic examination to be abnormal was examined histopathologically.

In the F_o generation, no intergroup differences in clinical signs were observed. One female in the F_o generation, receiving fenpyroximate at 30 ppm, was killed in extremis, its poor condition apparently being due to difficulties with parturition, this event occurring on the 23rd day post coitum. Although there were some minor intergroup differences in body-weight gain in the males of the F_o generation, these were not dose-related. In the females at 100 ppm, weight gain was reduced in the period up to pairing and during gestation. In the F_o females at 30 ppm, there was slightly reduced body-weight gain during gestation. In the F_o males at 100 ppm, there was slightly reduced food consumption compared with that of the controls, but food consumption was not reduced in males receiving fenpyroximate at at lower dietary concentration nor in any group of females. Estrus cycles, mating performance, gestation length and gestation index were not affected by treatment. At 100 ppm in the F_o generation, the conception rate and fertility index were slightly reduced. Litter size at birth and viability up until the time of weaning were not affected by treatment. The initial body weights of the offspring were similar in all groups, but weight gain until weaning of the F_1 offspring in the group receiving fenpyroximate at 100 ppm was reduced. Physical development in the groups was similar. There was no indication of a treatment-related effect on sex ratio. Necropsy of adult F_o rats, of F_1 offspring which died before weaning, and of F_1 offspring, which were culled on postnatal day 4 or which were not selected to be F_1 parents, did not show treatment-related macroscopic abnormalities. Absolute and relative weights of the reproductive organs in all groups were similar. No treatment-related effect was apparent on histopathological examination of the organs of reproduction.

One female in the F_1 generation receiving fenpyroximate at 100 ppm was killed in extremis on day 24 post coitum and was found to have a ruptured uterus. Intergroup differences in clinical signs were seen in neither sex. At 100 ppm, there was a reduction in body-weight gain in males of the F_1 generation. In females at 100 ppm, body-weight gain was reduced in the period up to pairing and during gestation, but recovered during lactation. Reduced body-weight gain was not seen at lower dietary concentrations in either sex. In the F_1 males at 100 ppm, food consumption was slightly reduced compared with that of the controls, but food consumption was not reduced in males receiving fenpyroximate at lower dietary concentrations, nor in any group of females. Estrus cycles, mating performance, gestation length and gestation index were not affected by treatment. Notably, at 100 ppm in the F_1 generation, the conception rate and fertility index were not reduced. Litter size at birth and viability up until the time of weaning were not affected by treatment. The initial body weights of the offspring were similar in all groups, but weight gain until weaning of the F_2 offspring in the group at 100 ppm was reduced. Physical development in the groups was similar. No intergroup differences in sex ratio, that were attributable to treatment, were seen. Necropsy of adult F_1 rats, of F_2 offspring that died before weaning and of F_1 offspring that were culled on postnatal day 4 or that were killed at termination after weaning did not show any treatment-related macroscopic abnormalities. In the F_1 males, the absolute weights

of the testes were increased in all treatment groups and the relative weights of the testes at 100 ppm. Epididymal weights (both relative and absolute) were increased at 100 ppm. Absolute and relative weights of other reproductive organs were not altered in a treatment-related fashion. No treatment-related effects were seen on histopathological examination of the organs of reproduction. The NOAELs for parental, offspring and reproductive toxicity were all 30 ppm (equal to 1.99 mg/kg bw per day for F_0 males, 2.44 mg/kg bw per day for F_0 females, 2.33 mg/kg bw per day for F_1 males and 2.82 mg/kg bw per day for F_1 females). The NOAEL for parental toxicity was identified on the basis of reduced body-weight gain and food consumption at 100 ppm, together with increased testicular and epididymal weights in males at 100 ppm. The NOAEL for offspring toxicity was identified on the basis of reduced body-weight gain in the offspring at 100 ppm, while that for reproductive performance was identified on the basis of reduction in conception rate and fertility index in the F_0 generation only (Higgins, 1989a).

(b) Developmental toxicity

Rats

In a study of developmental toxicity, groups of 22 pregnant CD rats were given fenpyroximate (purity, 97.6%) at a dose of 1, 5 or 25 mg/kg bw per day by gavage from day 6 to day 15 of gestation. A control group of rats received vehicle only. The rats were examined daily during the study and animals found dead were examined macroscopically. The rats were weighed on days 0, 3, 6 to 16, 18 and 20 of gestation. Food consumption and water consumption were recorded over days 0–2, 3–5, 6–8, 9–11, 12–15, 16–17 and 18–19 of gestation. The surviving rats were killed on day 20 of gestation for examination macroscopically and for examination of their uterine contents. The numbers of corpora lutea per ovary, implantation sites, resorption sites and number of live and dead fetuses were recorded. The fetuses were weighed, sexed and examined for external abnormalities. Approximately two thirds of the animals in each litter were processed and stained (with alizarin red) for skeletal examination and the remaining one third were fixed in Bouin's fixative and serially sectioned. Three deaths occurred on day 12 of gestation in the group receiving the intermediate dose (5 mg/kg bw per day) and none in the other groups; the cause of death could not determined, but because deaths were not observed in the group receiving the highest dose, the effect was not considered to be related to the test material. No intergroup differences in clinical condition were seen. At 25 mg/kg bw per day, there was initial weight loss and although weight gain thereafter was similar to that in the other groups, body weights in this group were persistently low compared with those of the controls. No intergroup differences in body weight or body-weight gain were seen in the other groups. Food intake at 25 mg/kg bw per day was initially reduced and water intake increased, but intergroup differences in food intake and water intake were not seen in the other groups. Necropsy at day 20 showed no treatment-related macroscopic abnormalities. The numbers of implantations, viable young and resorptions and the extent of pre- and postimplantation losses were not affected by treatment. Fetal and placental weights were unaffected by treatment. All treated groups showed increases in the number of thoracic ribs, which was greatest at 25 mg/kg bw per day. At the lower doses, there was no clear dose–response relationship. The NOAEL was accordingly 5 mg/kg bw per day on the basis of maternal weight loss, initially reduced maternal food consumption and increased maternal water intake. The NOAEL for embryo/fetal toxicity was also 5 mg/kg bw per day on the basis of increases in the number of thoracic ribs (Higgins, 1989b).

Rabbits

In a preliminary study of developmental toxicity, groups of four pregnant New Zealand white rabbits were given fenpyroximate (purity, 98.4%) at a dose of 2.5 or 5.0 mg/kg bw per day by gavage from day 6 to day 19 of gestation. An additional group of four pregnant rabbits received the vehicle only, and a group of three pregnant rabbits received fenpyroximate at a dose of 1.0 mg/kg bw per day by gavage. Animals were examined and weighed daily and adverse effects were recorded. Food and water intake was recorded over days 1–5, 6–12, 13–19, 20–23 and 24–28 of gestation. On day 29, the rabbits were killed, examined macroscopically and abnormal tissues were retained and fixed. The reproductive tract was dissected out and the number of corpora lutea in each ovary and the number of implantation sites and resorption sites were determined. Also, the number and distribution of live and dead fetuses were determined. The weight of each fetus and placenta was determined, and any external abnormalities of fetuses and placentas were recorded. The fetuses were eviscerated and one third were decapitated. Adverse effects were only seen at 5.0 mg/kg bw per day. These were depression of maternal body-weight gain and reduced food and water intake. Increased postimplantation loss, reduced fetal weight and anomalies were observed. The NOAELs for maternal and fetal toxicity were both 2.5 mg/kg bw per day (Bailey, 1989).

In a study of developmental toxicity, groups of 15 pregnant New Zealand white rabbits received fenpyroximate (purity, 97.6%) at a dose of 1.0, 2.5 or 5.0 mg/kg bw per day by gavage from day 6 to day 19 of gestation. An additional group of 15 pregnant rabbits received the vehicle only. The rabbits were observed daily throughout the study and any that were observed to be in extremis or that had aborted were killed, macroscopic examination being carried out. The rabbits were weighed daily and food and water intake was recorded for days 1–5, 6–12, 13–19, 20–23 and 24–28 days of gestation. On day 29 of gestation, the animals were killed and examined macroscopically. The reproductive tract, including the ovaries, were examined and the numbers of corpora lutea, implantation sites, resorption sites and live and dead fetuses were determined. The fetuses and placentas were weighed and examined for external abnormalities. Approximately two thirds of the animals in each litter were processed and stained (with alizarin red) for skeletal examination and the remaining one third were fixed in Bouin's fixative and serially sectioned. Two rabbits at 2.5 mg/kg bw per day group were killed in extremis. One appeared to have a respiratory infection and the other a gastrointestinal disturbance. There was a reduced level of faecal output in all the test groups, particularly in the group receiving the highest dose. Loss of body weight early in the treatment period followed by recovery was observed at 2.5 and 5.0 mg/kg bw per day. No effect on body weight was seen at 1.0 mg/kg bw per day. A reduction in food consumption was noted at 2.5 and 5.0 mg/kg bw per day and the reduction was marked at 5.0 mg/kg bw per day. A reduction in water consumption was seen at 5.0 mg/kg bw per day early in the study. One female in each of the groups receiving fenpyroximate at a dose of 2.5 or 5.0 mg/kg bw per day had complete litter resorption. The prevalence of complete litter loss was within the range for historical controls at the testing facility. One female receiving fenpyroximate at 5.0 mg/kg bw per day aborted. There were no intergroup differences in numbers of implantations, viable young, pre- and postimplantation loss and fetal and placental weights. A higher incidence of unilateral and bilateral slightly folded retinas was observed in the group at 5.0 mg/kg bw per day compared with the concurrent controls: the frequency of these findings was just within the ranges for controls at that testing facility (see Table 3). The biological significance of the higher incidence of unilateral and bilateral slightly folded retinas was unclear.

Table 3. Incidence (%) of slightly folded retina in fetuses and litters[a]

	Dose (mg/kg bw)			
	0	1	2.5	5
Unilateral	8.1 (3)	6.1 (2)	5.9 (2)	25.8 (6)
Bilateral	10.8 (3)	6.1 (2)	14.7 (4)	16.1 (5)

From King (1989)

[a] The first value is the percentage incidence in fetuses; the value in parentheses is the litter data

A single fetus at the highest dose had abnormalities of the heart and great vessels. The NOAEL for maternal toxicity was 1 mg/kg bw per day on the basis of reduced body weight and food consumption. The NOAEL for fetotoxicity was 2.5 mg/kg bw per day on the basis of unilateral and bilateral slightly folded retinas at 5 mg/kg bw per day. These NOAELs are consistent with those idientified in the preliminary study (King, 1989).

Comments

The acute oral LD_{50} of fenpyroximate was 245 and 480 mg/kg bw in male and female rats respectively.

The toxic effects of fenpyroximate include diarrhoea, failure to gain weight and haematological and clinical chemistry changes. In short-term (range-finding) dietary studies in mice, the effects of fenpyroximate were mainly limited to decreases in food consumption and reduced body-weight gain. The NOAELs for the two studies were 20 ppm (equal to 2.58 and 3.07 mg/kg bw per day in males and females, respectively) and 80 ppm (equal to 10.8 mg/kg bw per day in males and 11.7 mg/kg bw per day in females). In a 13-week dietary study in rats, effects were seen on body-weight gain, food consumption and haematological and clinical chemistry parameters. The NOAEL for the study was 20 ppm (equal to 1.30 mg/kg bw per day in males and 1.65 mg/kg bw per day in females). In a 13-week study in dogs given capsules containing fenpyroximate, weight loss or decreased weight gain, decreased food consumption and diarrhoea were seen. No NOAEL was identified in this study and the LOAEL was 2 mg/kg bw per day on the basis of diarrhoea occurring at all doses. This was considered to be a minimal effect level, and occurred from the beginning of the study. In a dietary study of reproductive toxicity in rats, parental toxicity comprised reduced body-weight gain and food consumption in both sexes, and increased testicular and epididymal weights in males. Offspring toxicity consisted of reduced body-weight gain. A reduction in conception rate and fertility index was observed in one generation only. The NOAELs for parental, offspring and reproductive toxicity were all 30 ppm (equal to 1.99 mg/kg bw per day in F_0 males, 2.44 mg/kg bw per day in F_0 females, 2.33 mg/kg bw per day in F_1 males and 2.82 mg/kg bw per day in F_1 females). In a study of developmental toxicity in rats treated by gavage, effects were seen on maternal body weight, while increases in the number of thoracic ribs were found in fetuses. The NOAEL for both effects was 5 mg/kg bw per day. In preliminary and substantive studies of the developmental toxicity of fenpyroximate in rabbits treated by gavage, reduced maternal body weight and food consumption was seen at the higher doses. These effects were not considered relevant for establishing an ARfD. The NOAEL for maternal toxicity was 1 mg/kg bw day. Significant elevations in the frequency of unilateral and bilateral slightly folded retinas were observed in fetuses at 5 mg/kg bw per day. No other significant evidence of fetotoxicity was observed and the biological significance of this finding is unclear.

Toxicological evaluation

The Meeting established an ARfD of 0.01 mg/kg bw on the basis of the a minimal LOAEL of 2 mg/kg bw per day for the induction of diarrhoea at the beginning of a 13-week study of toxicity in dogs. It was unclear whether the diarrhoea was the result of a direct irritant or pharmacological effect of fenpyroximate. A safety factor of 200 was used since no NOAEL was identified. This ARfD is probably conservative and could be refined using the results of an appropriately designed study.

Estimate of acute reference dose

0.01 mg/kg bw

Studies that would provide information useful for continued evaluation of the compound

Appropriately designed single-dose study

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Rat	Two-generation study of reproductive toxicity[a]	Parental toxicity	30 ppm, equal to 1.99 mg/kg bw per day	100 ppm, equal to 6.59 mg/kg bw per day
		Reproductive toxicity	30 ppm, equal to 1.99 mg/kg bw per day	100 ppm, equal to 6.59 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	5 mg/kg bw per day	25 mg/kg bw per day
		Embryo and feto-toxicity	5 mg/kg bw per day	25 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	1 mg/kg bw per day	2.5 mg/kg bw per day
		Embryo and feto-toxicity	2.5 mg/kg bw per day	5 mg/kg bw per day
Dog	13-week study of toxicity[c,d]	Toxicity	—	2 mg/kg bw per day[e]

[a] Diet
[b] Gavage
[c] Capsules
[d] Effects evident within 1 week
[e] Lowest dose tested

References

Aughton, P. (1987) NNI-850: toxicity study by dietary administration to CD rats for 13 weeks. Unpublished report No. 89/NNH021/0972 from Life Sciences Research, Eye, Suffolk, England. Submitted to WHO by Nihon Nohyaku Co. Ltd, Tokyo, Japan. GLP: USA, USEPA (40 CFR 160); UK DHSS; OECD (1981); MAFF, Japan.

Bailey, G.P. (1989) NNI-850: preliminary teratology study in the rabbit. Unpublished report No. 89/NNH050/0981 amending report 89/NNH050/0393 from Life Sciences Research, Eye, Suffolk, England. Submitted to WHO by Nihon Nohyaki Co. Ltd, Osaka, Japan. GLP: USA, USEPA (40 CFR 160); UK DHSS; OECD (1981); MAFF, Japan.

Broadmeadow, A. (1989) NNI-850: toxicity study by oral (capsule) administration to CD beagle dogs for 13 weeks. Unpublished report No. 89/NNH036/1111 (amended from 89/NNH036/614) from Life Sciences Research, Eye, Suffolk, England. Submitted to WHO by Nihon Nohyaku Co. Ltd, Tokyo, Japan. GLP: USA, USEPA (40 CFR 160); UK DHSS; OECD (1981); MAFF, Japan. Guidelines: USEPA/FIFRA, 1982; MAFF Japan, 1985.

Higgins, C. (1989a) NNI-850: reproductive performance study in rats treated continuously through successive generations. Unpublished report No. 90/NNH043/0141 from Life Sciences Research, Eye, Suffolk, England. Submitted to WHO by Nihon Nohyaki Co. Ltd, Osaka, Japan. GLP: USA, USEPA (40 CFR 160); UK DHSS; OECD (1981); MAFF, Japan.

Higgins, C. (1989b) NNI-850: teratology study in the rat. Unpublished report No. 89/NNH053/0722 from Life Sciences Research, Eye, Suffolk, England. Submitted to WHO by Nihon Nohyaki Co. Ltd, Osaka, Japan. GLP: USA, USEPA (40 CFR 160); UK DHSS; OECD (1981); MAFF, Japan.

King, V.C. (1989) NNI-850: teratology study in the rabbit. Unpublished report No. 89/NNH051/0687 from Life Sciences Research, Eye, Suffolk, England. Submitted to WHO by Nihon Nohyaki Co. Ltd, Osaka, Japan. GLP: USA, USEPA (40 CFR 160); UK DHSS; OECD (1981); MAFF, Japan.

Takahashi, K. (1987) NNI-850: 18-month oral oncogenicity study in mice 4 week dose range finding study. Unpublished report No. IET 87-0034 from Institute of Environmental Toxicology, Kodaira-shi, Tokyo, Japan. Submitted to WHO by Nihon Noyaku Co. Ltd, Tokyo, Japan. GLP: USA, EPA (40 CFR 160) (1983); OECD (1981); MAFF, Japan (1984).

Takahashi, K. (1988) NNI-850: 18-month oral oncogenicity study in mice 4 week dose range finding study. Unpublished report No. IET 87-0186 from Institute of Environmental Toxicology, Kodaira-shi, Tokyo, Japan. Submitted to WHO by Nihon Noyaki Co. Ltd, Tokyo, Japan. GLP: USA, FIFRA (1983); OECD (1981); MAFF, Japan (1984).

FLUDIOXONIL

First draft prepared by
A. Bartholomaeus
Therapeutic Goods Administration, Canberra, Australia

Explanation

Fludioxonil is the International Organization for Standardization (ISO) approved name for a new phenylpyrrole fungicide, 4-(2,2-difluoro-1,3-benzodioxol-4-yl)pyrrole-3-carbonitrile (International Union of Pure and Applied Chemistry, IUPAC), that interferes with glucose transport across fungal membranes. Fludioxonil has not been evaluated previously by the JMPR.

Evaluation for acceptable daily intake

Unless otherwise stated, the studies evaluated in this monograph were performed by laboratories that were certified for good laboratory practice (GLP) and complied with the relevant Organisation for Economic Co-operation and Development (OECD) test guideline(s) or similar guidelines of the European Union or United States Environmental Protection Agency. As these guidelines specify the tissues normally examined and the clinical pathology tests normally performed, only significant exceptions to these guidelines are reported here, to avoid repetitive listing of study parameters.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Rats were treated by gavage with [^{14}C]pyrrole fludioxonil (purity, 99%), which was supplemented with unlabelled fludioxonil (purity, 99%) at the highest dose. Groups of five male and five female rats were given a single dose of [^{14}C]fludioxonil at 0.5 mg/kg bw, or

Figure 1. Chemical structure of fludioxonil

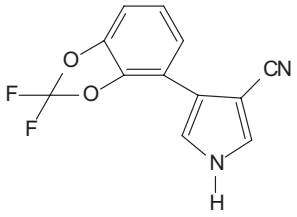


Table 1. Excretion of radiolabel (% of administered dose) in rats 7 days after oral treatment with fludioxonil

Excreta	Dose					
	0.5 mg/kg bw, single dose		0.5 mg/kg bw per day, for 14 days		100 mg/kg bw, single dose	
	Males	Females	Males	Females	Males	Females
Urine	16.2	16.9	13.4	14.6	16.8	19.5
Faeces	81.2	79.1	82.8	81.5	77.6	77.6
Air	—	—	—	—	<0.01	<0.01

From Bissig (1990)

were pretreated with unlabelled fludioxonil for 14 days and then given a single dose of [^{14}C]fludioxonil at 0.5 or 100 mg/kg bw. Urine and faeces and, at 100 mg/kg bw, expired air were collected over the subsequent 7 days, after which tissues were analysed for radiolabel. An additional group of three females was treated with [^{14}C]fludioxonil at 0.5 mg/kg bw and blood was collected over the subsequent 48 h. An additional group of 10 females was treated with [^{14}C]fludioxonil at 0.5 mg/kg bw and tissues were collected after either 0.5 h (t_{cmax}) or 9 h ($t_{cmax/2}$). Another group of five bile-duct cannulated females was treated with [^{14}C] fludioxonil at 100 mg/kg bw and urine, faeces and bile were collected over the next 48 h. In bile-duct cannulated rats, approximately 67% of the administered dose was excreted in the bile, 10% in the urine and 14% in the faeces over the first 48 h, with at least 77% of the administered dose being absorbed from the intestinal tract. In intact animals, faecal excretion was of the order of 80% of the administered dose. Blood kinetic investigations demonstrated rapid absorption of an oral dose of 0.5 mg/kg bw from the intestinal tract into the general circulation, with maximum blood concentration (t_{cmax}) reached within 0.5 h, declining to half this concentration after 9 h ($t_{cmax/2}$). Rate and route of excretion were independent of both sex and dose. Approximately 96% of the administered dose was excreted within 7 days. Most of this was excreted within the first 24 h. Seven days after a single oral dose of [^{14}C]fludioxonil at 0.5 mg/kg bw, in rats that had or had not been pretreated with 14 daily oral doses of unlabelled fludioxonil at 0.5 mg/kg bw per day, concentrations of radiolabel in most tissues were below the limit of detection. Fludioxonil equivalents were detected in the liver (0.002 ppm), kidneys (0.004 ppm), blood (0.0007 ppm) and lungs (0.0012 ppm). After a dose of 100 mg/kg bw, tissue residues other than in the liver, kidneys, blood and lungs were low, but concentrations in the tissues of females were generally twice those found in males. The total amount of radioactivity recovered in tissues was approximately 0.17% of the administered dose. Elimination of radiolabelled fludioxonil from tissues ($t_{1/2}$) was biphasic; between 2 and 6 h for phase 1 and between 30 and 60 h for phase 2 (Bissig, 1990).

Groups of three rats were treated with single doses of ^{14}C-labelled fludioxonil at 0.5 or 100 mg/kg bw by gavage and blood was collected for 48 h. Additional groups were treated

at the same doses and sacrificed for analysis of tissues at t_{cmax}, $t_{cmax/2}$ and two other time-points. At 0.5 mg/kg bw, maximum blood concentrations (fludioxonil equivalents: males, 0.065 mg/l; and females, 0.027 mg/l) were achieved after 15 min. Biphasic elimination was observed, with a rapid first phase and slow second phase. Blood concentrations declined to half C_{max} within 1 h. At 100 mg/kg bw, maximum blood concentrations (fludioxonil equivalents: males, 4.5 mg/l; and females, 3.2 mg/l) were achieved between 4 and 8 h. Concentrations again declined in a biphasic manner reaching half C_{max} within a further 8 h. The area under the curve of concentration–time (AUC) was approximately proportional to the dose administered and was similar in males and females. Thus, the proportion of a dose absorbed was independent of both sex and dose. At 0.5 mg/kg bw at t_{cmax}, all residues contained fludioxonil equivalents at ≤1 ppm and most were <0.1 ppm, except in the liver, kidneys, lungs and plasma in all animals and blood and heart in females. The distribution pattern was similar at the highest and lowest doses. At the highest dose, residues were <13 mg of fludioxonil equivalents per kg at t_{cmax}. Tissue depletion kinetics were biphasic for both doses. As fludioxonil is rapidly cleared from both the blood and the tissues, there appears to be little potential for bioaccumulation of either unchanged fludioxonil or its metabolites (Müller & Thanei, 1995).

Fludioxonil (purity, 97%) labelled with ^{14}C in the phenyl ring and unlabelled fludioxonil (purity, 99.9%), in a wettable granule formulation (62.5 g/kg), diluted in water, was applied to the shaved skin (10 cm^2) of male rats (body weight, approximately 250 g) at a concentration of 4.5 µg/cm^2 (reflecting a typical concentration recommended for application in the field) or at 560 µg/cm^2 for an exposure period of 6 h. After exposure, the application site was washed three times with a mild soap solution. The depletion of the remaining radioactivity at the application site, after washing, was determined at 24 and 48 h after application. Urine and faeces were collected from animals individually at 0–6, 6–24 and 24–48 h after application. For four animals at each dose, blood was collected at 0.5, 1, 2, 4, 6, 8, 24 h and 48 h after application by cutting the tip of the tail. At sacrifice, the amount of radiolabel was measured in the blood, urine, faeces, cage wash, skin wash, at the application site, in control skin, extracts of the cover and residual carcass. At 4.5 µg/cm^2, blood radioactivity was below the limit of detection at all time-points and absorption was low, reaching 5.1% at 24 h and 7.4% of the applied dose at 48 h after application. Elimination was mainly in the faeces (3.78% and 5.78% at 24 h and 48 h, respectively) and to a lesser degree in the urine (0.69% and 1.01%, respectively). At 560 µg/cm^2, the concentration of radiolabel in the blood reached a maximum of 0.017 mg of fludioxonil equivalents/l at 0.5 h after application and decreased rapidly thereafter to below the limit of detection at 1 h and all following sampling points. Absorption was lower than that at the lowest dose, 0.32% of the applied dose after 24 h and 0.61% after 48 h, reflecting a saturation of the rate of dermal flux. The bulk of the applied doses (76.5–90.5%) was removed with the skin wash at 6 h after application. The amount of fludioxonil remaining in the skin at 6, 24 and 48 h was almost constant (6.0, 8.5 and 5.7% at 4.5 µg/cm^2; and 3.1, 4.3 and 2.7% at 560 µg/cm^2) indicating little mobility of fludioxonil in the skin depot. Assuming that a steady state for percutaneous penetration is rapidly attained and maintained during the first 6 h after application, the flux rate at 560 µg/cm^2 (0.0774 µg/cm^2 · per h) was more than three times that at 4.5 µg/cm^2 (0.0284 µg/cm^{-2} · per h), despite the dose being 120-fold greater, indicating saturation of the rate of dermal flux at the highest dose. Although a proportion of the fludioxonil remaining in the skin 24 h after application is available for subsequent movement into the systemic circulation, for human risk assessment purposes the appropriate dose metric is the amount reaching the systemic circulation during 24 h, as it is this amount that can reasonably be compared with appropriate no-observed-adverse-effect levels (NOAELs).

Table 2. In vivo percutaneous absorption/excretion of radiolabel (% of applied dose) in rats treated with fludioxonil

Dose	4.5 µg/cm²			560 µg/cm²		
Sacrifice time (h)	6	24	48	6	24	48
Total excretion (urine, faeces, cage wash)	0.21	4.5	6.84	0.01	0.18	0.49
Residues (whole blood, non-treated skin, carcass)	3.41	0.64	0.53	0.07	0.14	0.12
Total systemic absorption	3.6	5.1	7.4	0.08	0.32	0.6
Treated skin	6.0	8.5	5.7	3.1	4.33	2.7

From Mewes (1999)

On this basis the dermal absorption factors for fludioxonil are approximately 5 and 0.5% of the applied dose at exposures of 4.5 and 560 µg/cm² respectively (Mewes, 1999).

Fludioxonil (purity, 99%) labelled with ^{14}C in the phenyl ring and unlabelled fludioxonil (99.8%), in a wettable granule formulation (62.5 g/kg), diluted in water, was applied at a concentration of 3.6 µg/cm² to rat skin or at a concentration of 3.4 µg/cm² to human skin (these valuses reflecting a typical concentration recommended for application in the field—300 g of active ingredient/800 l per 10 000 m²) and at 690 µg/cm² to rat skin and at 720 µg/cm² human skin for 6 h, without occlusion. Rat epidermis was prepared from male rats aged approximately 23 days, and human cadaver abdominal epidermal skin was obtained from Caucasian donors (one male, aged 68 years, and one female, aged 86 years). Circles of epidermal membranes (approximately 1.8 cm in diameter) were cut and mounted in the diffusion cells between the donor and receptor chamber. The receptor fluid was ethanol/water (1 : 1 v/v) delivered at a flow rate of about 3 ml/h. Perfusates were collected hourly for the first 6 h after application, then every 2 h until 48 h. The penetration of fludioxonil through human skin was markedly lower than through rat skin (1% to 2% of the values in rats).

For rat skin, the flux, which reflects the rate of penetration under steady-state conditions, amounted to 0.075 µg/cm²/h at the lowest dose (steady-state conditions from about 1 h to 19 h). At the highest dose, the flux was 0.52 µg/cm² per h (steady-state conditions from about 1 h to 48 h). With the human epidermis, the majority of the individual values determined in the perfusate were below the limit of determination (LQ). However, based on the determined values, 0.2% and 0.01% of the applied dose penetrated the epidermis at the lowest and highest dose within 12 h, corresponding to 0.01 and 0.08 µg/cm² The total penetration at the two doses was 0.5% and 0.02% of the applied dose within 24 h. Since the individual values were mainly below the limit of determination, the flux constants were calculated based on the corresponding LQ values accounting for 0.0007 and 0.008 µg/cm² per h at the lowest and highest dose, respectively (Hassler, 1999).

1.2 Biotransformation

Pooled urine, bile and faeces collected from animals given ^{14}C-labelled fludioxonil in a previous kinetic study (Bissig, 1990) were used to investigate the metabolic transformation of fludioxonil. No unchanged fludioxonil was detected in the urine or bile. Seven metabolites were detected in the bile. Four metabolites were separated and identified. Two of the metabolites were glucuronic acid derivatives of fludioxonil, with one of these being the major metabolite (56% of the administered dose). Twenty metabolites were detected in

Table 3. Penetration of rat and human epidermis by fludioxonil

	% of administered dose	Flux (μg/cm²)	% of administered dose	Flux (μg/cm²)
Rat epidermis				
Dose administered	3.6 μg/cm²		687 μg/cm²	
12 h	23.91[a]	0.86[a]	0.69	4.72
24 h	37.67[a]	1.35[a]	1.35	9.72
48 h	50.73[a]	1.82[a]	2.69	18.47
Flux rate (μg/cm² per h)	0.075		0.518	
Human epidermis				
Dose administered	3.4 μg/cm²		718 μg/cm²	
12 h	0.24[b]	0.01[b]	0.01[b]	0.08[b]
24 h	0.50[b]	0.02[b]	0.02[b]	0.16[b]
48 h	1.03[b]	0.03[b]	0.05[b]	0.33[b]
Flux rate (μg/cm² per h)	0.0007[c]		0.008[c]	

From Hassler (1999)

[a] These values were calculated based on an interpolation of the corresponding mean values of 11–13 h and 23–25 h and an extrapolation of the values at 47 h

[b] Most of the individual values are below the limit of determination

[c] Flux calculated based on the corresponding LQ values

Table 4. Metabolites in rats given a single oral dose of fludioxonil at 100 mg/kg bw

Metabolite	Chemical name	% of administered dose (urine, faeces, and bile)[a]
SYN 518577	4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-2-hydroxy-1*H*-pyrrole-3-carbonitrile	0
Met 1G (SYN518577 glucuronyl conjugate	2-β-D-glucuronyl-4–(2,2-difluoro-benzo[1,3]dioxol-4-yl)-1*H*-pyrrole-3-carbonitrile	56.0 (0.5, 0, 55.5)
Met 2G (SYN518577 sulfate conjugate)	4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-1*H*-pyrrole-3-carbonitrile-2-hydrogen sulfate	<0.7 (<0.5, 0, <0.2)
SYN 518578	4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-5-hydroxy-1*H*-pyrrole-3-carbonitrile	0
MET 3G (SYN 518578 glucuronyl conjugate)	4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-5-β-D-glucuronyl-1*H*-pyrrole-3-carbonitrile	2.8 (0.7, 0, 2.1)
MET 1U (SYN 518578 sulfate conjugate)	4-(2,2-difluoro-benzo[1,3]dioxol-4-yl)-1*H*-pyrrole-3-carbonitrile-5-hydrogen sulfate	1.1 (1.1, 0, 0)
SYN 518576	4-(2,2-difluoro-7-hydroxy-benzo[1,3]dioxol-4-yl)-1*H*-pyrrole-3-carbonitrile	0
MET 4G (SYN 518576 glucuronyl conjugate)	4-(2,2-difluoro-7–β-D-glucuronyl-benzo[1,3]dioxol-4-yl)-1*H*-pyrrole-3-carbonitrile	2.2 (0.5, 0, 1.7)
Fludioxonil (unchanged)		11.6 (0, 11.6, 0)

From Thanei (1992)

[a] Percentage of the administered dose found in all samples combined; in parentheses, % of the administered dose that was found in the urine, faeces, and bile, respectively

the urine; four of these were identical to metabolites in the bile. The predominant radiolabelled substance in the faeces (2–11% of the administered dose) was unchanged fludioxonil. The metabolism of fludioxonil was extensive, involving primarily oxidation of the pyrrole ring leading to a major (57% of the administered dose) and a minor (4% of the administered dose) oxo-pyrrole metabolite; hydroxylation of the phenyl ring yielded the corresponding phenol metabolite, representing 2% of the administered dose. These phase I metabolites were subsequently excreted as glucuronyl- and sulfate conjugates, and, together with unabsorbed and unchanged fludioxonil excreted in the faeces, accounted for approximately 75% of the administered dose. Investigation of a blue metabolite of fludioxonil is discussed further in section 2.6 (Thanei, 1992).

Table 5. Distribution of metabolites in rats given a single oral dose of fludioxonil at 100 mg/kg bw

Metabolite[a]	Fludioxonil equivalents (% of administered dose)			
	Urine	Faeces	Bile	Total
MET 1G	0.5	—	55.5	56.0
MET 2G	<0.5	—	<0.2	<0.7
Total conjugates of SYN 518577				about 56.7
MET 3G	0.7	—	2.1	2.8
MET 1U	1.1	—	—	1.1
Total conjugates of SYN 518578				3.9
MET 4G (conjugate of SYN 518576)	0.5	—	1.7	2.2
CGA 173506 (fludioxonil)	—	11.6	—	11.6
Coloured	0.4	—	—	0.4
Total	3.2–3.7	11.6	59.3–59.5	74.1–74.8

From Thanei (1992)
[a] See Figure 2 for structures associated with the codes for metabolites in this table

Table 6. Acute toxicity of fludioxonil

Species[b]	Strain	Route	LD_{50} (mg/kg bw)/LC_{50} (mg/l)	Reference
Mouse	Hsd:ICR	Oral	>5000; no deaths	Glaza (1991c)
Rat	Hsd:Sprague-Dawley	Oral	>5000; no deaths	Glaza (1991a)
Rat	Tif:RAIf	Dermal	>2000; no deaths	Hartmann (1988)
Rat	Tif:RAIf	Inhalation	>2.6; no deaths[a]	Hartmann (1989)
Rat	Hsd:Sprague-Dawley	Inhalation	>0.54; no deaths	Holbert (1992)

[a] Piloerection, hunched posture and dyspnoea were noticed for up to 7 days
[b] All values are for both sexes combined

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

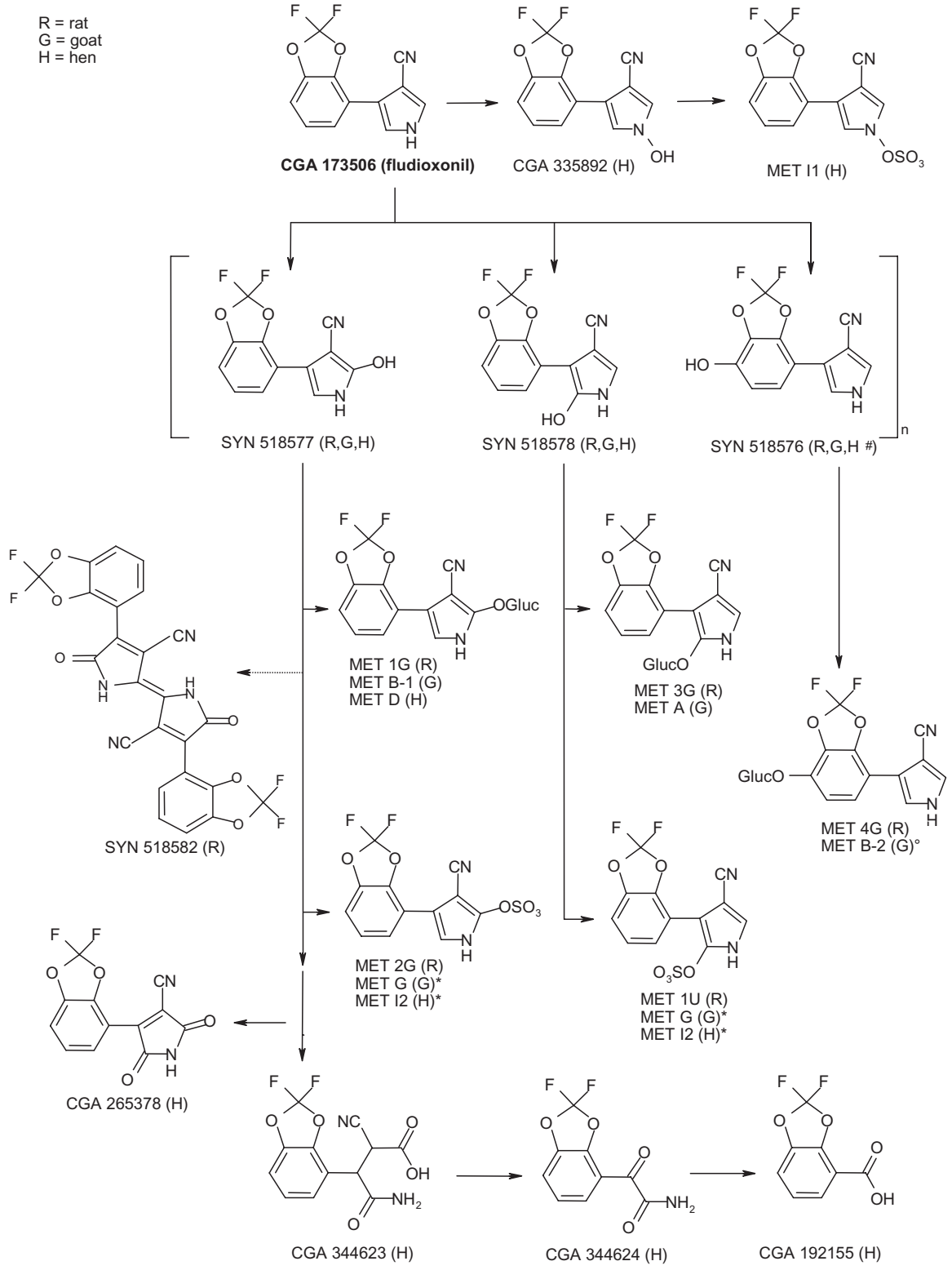
Table 6 summarizes the results of studies to establish the acute toxicity of fludioxonil.

(b) Dermal and ocular irritation and dermal sensitization

In two studies, New Zealand white rabbits were exposed dermally to 0.5 g of fludioxonil (purity, 97.5%) under a semi-occlusive dressing moistened with water or 0.9% saline. No deaths occurred, and no effects on body weights or clinical signs were noted. In the first study, erythema (grade 1) that persisted for up to 48 h was noted in two animals. Grade 1 oedema was also noted in one animal 1 h after removing the bandage. The mean irritation score for 24 to 48 h was 0.66 (Schneider, 1988a). In the second study there were no signs of erythema or oedema (Glaza, 1991b). Fludioxonil is not an irritant to rabbit skin.

Fludioxonil (purity, 97.5%), 0.1 ml, was placed in the left eye of three female rabbits. The lids were held closed for 1 s. The right eye remained untreated and served as a control. Slight conjunctival redness was seen in three animals at 1 h; this had regressed in one animal by 24 h, and by 48 h in the other two animals. Chemosis (grade 1) of the conjunctiva was noted in one animal at 1 h, clearing by 24 h. No effects on the cornea or iris were noted. Fludioxonil was at most a slight eye irritant in female rabbits (Schneider, 1988b).

Figure 2. Comparative metabolism of fludioxonil in rats, goats and hens



* no differentiation was made in hens and goats concerning 2-or 5-position of the conjugate
# isolated in hens; intermediate in rats and goats
° tentative identification in goats

Fludioxonil (95.4%, 0.1 ml) was placed in the left eye of New Zealand white rabbits and the lids were held closed for 1 s. Three rabbits had the test materials flushed from the treated eye with lukewarm tap water for 60 s, 30 s after exposure. The right eye remained untreated and served as a control. No corneal damage was observed. In unwashed eyes, iridial irritation (grade 1) was seen at 1 h, continuing for up to 48 h, conjunctival redness (grade 2–3) was seen at 1 h decreasing in severity over the next 72 h and chemosis (grade 1–3) was noted in all eyes at 1 h decreasing in severity over the next 48 h. A clear discharge of the conjunctivae (grade 2) was noted in one animal at 1 h. In the washed eyes, irritation of the iris was noted in one eye 1 h after exposure (grade 1), conjunctival redness (grade 2) in all animals at 1 h and in two animals at 48 h, and chemosis (grade 1) in all eyes at 1 h persisting in one animal for 24 h. Fludioxonil caused slight ocular irritation in rabbits. Washing the eye immediately with water reduced the severity of the irritation. Average irritation scores for unwashed/washed eyes at 1, 24, 48, 72 and 96 h respectively were; 13.7/7.7, 6.8/2.7, 3.7/1.3, 0.7/0, 0/0 (Glaza, 1991d).

In a Magnussen & Kligman maximization assay, Pirbright white guinea-pigs were given 0.1 ml of fludioxonil (purity, 97.5%) by intradermal injection into the shaved neck. One week later, a second induction dose was applied topically by incorporation in vaseline onto a patch and placed on the neck. The exposed region was covered with an occlusive dressing for 48 h. On the day before this treatment, the application sites were pretreated with 10% sodium lauryl sulfate. Two weeks later, fludioxonil in vaseline was applied under an occlusive dressing to the flank for 24 h. Control animals were treated with the vehicle only during the induction period and exposed to fludioxonil in the challenge phase. The doses of fludioxonil used were: intradermal induction, 1%; epidermal induction, 30%; epidermal challenge, 10%. Skin reactions were evaluated after the induction doses and 24 and 48 h after removing the challenge dressing. Body weights were recorded at the start and end of the study. Food, water and the housing environment were controlled and monitored. No signs of irritation were noted for control or test animals after the induction period. No signs of irritation were noted after the challenge dose for either control or test animals. There was no apparent affect on body weight. Fludioxonil did not cause skin sensitization in guinea-pigs (Schneider, 1988c).

2.2 Short-term studies of toxicity

Mice

Groups of 10 male and 10 female CD-1 mice were fed diets containing fludioxonil (purity, 96%) at a concentration of 0, 10, 100, 1000, 3000 or 7000 ppm for 90 days. Actual fludioxonil intakes were 0, 1.3, 14, 140, 450 or 1050 mg/kg bw per day for males and 0, 1.9, 17, 180, 560 or 1310 mg/kg bw per day. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, body weight and food consumption measurements, ophthalmology, haematology (not including mean cell haemoglobin concentration or prothrombin time), serum biochemistry (including measurements of direct bilirubin, 5′nucleotidase and sorbital dehydrogenase activty), urine analysis, organ weights, gross pathology and histopathology (including thigh muscle).

There were no deaths and no treatment-related ophthalmological changes. Haematology parameters were unaffected and clinical signs were limited to discoloured urine and blue staining of the pelvic region at 1000, 3000 and 7000 ppm in males. Significantly lower body-weight gains were noted in females at 7000 ppm (73% of control) throughout the study.

Table 7. Key findings in a 90-day study in mice given diets containing fludioxonil

Finding	Sex (historical control range)	Dietary concentration (ppm)					
		0	10	100	1000	3000	7000
Serum biochemistry							
Potassium (mg/dl)	Males (3.8–5.1)	4.8	4.6	4.5	4.5	4.4	4.7
	Females (3.7–5.3)	4.8	4.6	4.7	4.7	4.5	3.9**
5′Nucleotidases (IU/l)	Males (10–21)	14	15	13	13	15	18**
	Females (25–53)	33	33	31	35	39	50**
Organ weights							
Liver, absolute (g)	Males	1.455	1.446	1.387	1.483	1.437	1.613
	Females	1.225	1.172	1.217	1.245	1.324	1.375**
Liver, relative to body weight	Males	4.044	3.890	3.981	4.106	4.205	4.745**
	Females	4.166	4.178	4.074	4.283	4.749*	5.065**
Thymus, absolute (g)	Males	0.023	0.027	0.023	0.023	0.019	0.022
	Females	0.033	0.027	0.032	0.027	0.028	0.022*
Thymus, relative to brain weight	Males	4.730	5.670	4.712	4.544	3.802	4.503
	Females	6.524	5.371	6.461	5.449	5.484	4.474*
Pathology							
Kidneys, nephropathy	Males	2	2	2	2	1	10**
	Females	2	3	1	2	2	9**
Liver, hypertrophy	Males	0	0	0	0	0	7**
	Females	0	0	0	0	3	8**

From Chang & Morrissey (1990); Chang (1990b)
*$p < 0.05$, **$p < 0.01$

Body weights in males and food intake in both sexes were unaffected. The concentration of 5′nucleotidase in serum was significantly increased in mice at 7000 ppm. Females exposed to fludioxonil at 7000 ppm had significantly reduced concentrations of potassium. These variations are within the range for historical controls. Discolouration of the urine, due to the presence of a coloured metabolite, and the presence of bilirubin was noted in males at 1000, 3000 and 7000 ppm and discolouration of the kidneys and digestive tract was noted in males and females at 7000 ppm. Liver weights, absolute and relative to body weight, were increased in mice of both sexes at 7000 ppm and correlated with centrilobular hypertrophy in females at 3000 ppm and in both sexes at 7000 ppm. In the absence of clinical chemistry reflecting liver damage, and as these liver changes are likely to reflect an adaptive response to the high xenobiotic load, it was concluded that they were not adverse effects. Thymus weights, both absolute and relative to brain weight, were significantly decreased in females at 7000 ppm. The incidence of chronic nephropathy was increased in mice of both sexes at 7000 ppm. The NOAEL for this study was 3000 ppm (450 mg/kg bw per day) on the basis of nephropathy and elevated serum 5′nucleotidase activity in both sexes and reduced weight gains in females at 7000 ppm (Chang & Morrissey, 1990; Chang, 1990b).

Rats

In a range-finding study, groups of six male and six female Sprague Dawley Crl: CD(SD)Br rats received diets containing fludioxonil (purity not reported) at a concentration of 0, 1000, 5000, 10 000 or 20 000 ppm (equal to 120, 620, 1300 or 2500 mg/kg bw per day for males and 130, 700, 1400 or 2800 mg/kg bw per day for females) for 20 days (males) or 21 days (females). The homogenicity and stability of fludioxonil in the diet was checked before the study. Mortality was checked twice per day on working days and once on weekends. Clinical signs were checked daily. Body weight, and food (three animals per group) and water consumption (three animals per group) were recorded weekly. At the end of treatment, all animals were subjected to the following examinations: haematology (in four to six animals of each group; reticulocyte counts were not performed, and differential leukocyte

count was carried out only for animals in the control group and at the highest dose), clinical chemistry (except phospholipids, bilirubin, creatinine, creatinine kinase), urine analysis (except specific gravity and examination of sediment). At sacrifice, the adrenals, brain, heart, kidneys, liver, spleeen, thymus, ovary and testes were weighed and the adrenals, heart, kidneys, liver, spleen and thymus were examined histologically (for animals in the control group and at the highest dose; kidneys were also examined at the intermediate doses).

There were no mortalities. Clinical signs were limited to black faeces in animals treated with fludioxonil at ≥5000 ppm during the last week of treatment, which may have been due to the presence of the blue-coloured metabolite SYN 518582 formed by non-enzymatic autoxidation at high doses. This effect is not considered to be toxicologically relevant. In males at 20 000 ppm, body weights at the end of the treatment period were 88% of those of the controls, food consumption was 90% of that of the controls and water consumption was increased by approximately 20% (also at 10 000 ppm). Haematological and urine analysis parameters were unaffected by treatment, although individual males at 10 000 and 20 000 ppm had slightly lower urine volumes with a slightly increased specific gravity. A slight (≤5%) but highly statistically significant reduction in concentrations of sodium and chloride in both sexes at ≥5000 ppm appears to be treatment related, and correlates with tubular nephrosis, although similar findings were not obtained in any other studies of repeated dose in rats, including the 28-day study, reported below, which used comparable doses. A dose-related increase in cholesterol levels was seen in both sexes, which wsaa significant at 20 000 ppm only. A slight decrease in concentrations of glucose was seen in females at ≥10 000 ppm. No toxicological or biological relevance was attributed to the variations in α-1 globulins in the males as a dose–response relationship was not apparent and a similar effect was not seen in females. Slight decreases in alkaline phosphatase and alanine aminotransferase activities, while likely to be treatment related, were discounted as not being adverse. Alkaline phosphatase and alanine transferase serum activities in rats are affected by nutritional status and are commonly found to be lower in rats gaining less weight and/or eating less food.

Increased absolute and relative weights of the kidney were observed at ≥10 000 ppm and where associated with tubular nephrosis. In addition, paleness of the kidneys and blackish and punctiform foci were seen in one to two males at 10 000 and 20 000 ppm. A single male at 5000 ppm also had tubular nephrosis. Increased relative weights of the liver were observed at 10 000 and 20 000 ppm, but were not associated with any clinical chemistry or histopathological changes consistent with liver damage and were therefore considered to be likely to reflect an adaptive response. Further variations in the absolute and/or relative weight of some organs (heart, spleen, brain) were not associated with any histopathological changes (heart, spleen), considered to be secondary to the reduced body weights for males at 20 000 ppm (increased relative brain weights, reduced absolute heart and spleen weight) and/or without a dose–response relationship (heart and spleen weights in females).The NOAEL was 1000 ppm, equal to 120 and 130 mg/kg bw per day in males and females respectively, on the basis of altered kidney histology at higher doses (Courcy di Rosa, 1988).

Groups of 10 male and 10 female Tif: RAIf rats were given fludioxonil (purity, 97.5%) at a dose of 0, 10, 100 and 1000 mg/kg bw per day by gavage in distilled water containing 0.5% carboxymethylcellulose and 0.1% Tween 80, daily for 28 days. Food, water and the housing environment were controlled and monitored. Toxicity was evaluated through: mortality, daily clinical observation, body weight, measurements of food and water con-

Table 8. Clinical pathology, organs weights and histopathological changes in 20-day study in rats given diets containing fludioxonil

	Dietary concentration (ppm)									
	Males					Females				
	0	1000	5000	10 000	20 000	0	1000	5000	10 000	20 000
Clinical pathology										
Cl^- (mmol/l)	107.8	109.4	103.6**	104.5*	103.0**	103.7	104.3	100.4**	98.3**	101.0**
Na^+ (mmol/l)	142.3	142.9	138.8**	137.9**	138.0**	136.9	138.2	133.2**	133.4**	133.5**
Glucose (mmol/l)	5.34	6.54	4.89	6.33	5.10	5.87	5.69	5.36	4.78*	4.59**
Cholesterol (mmol/l)	1.9	2.2	2.3	2.5	2.7*	2.3	2.2	2.4	2.5	3.2**
Alkaline phosphatase (U/l)	563	472	421	417	424	382	397	313	265**	333
Alanine transferase (U/l)	27	21*	20*	21	18**	25	24	21	20	18*
α-1 Globulin (g/l)	4.3	2.5**	2.6**	2.7**	3.1*	1.8	2.5	1.9	2.4	1.7
Organ weights and histology										
Carcass (g)	249	238	242	231	202**	159	156	149	161	149
Kidney:										
Absolute weight (g)	2.274	2.227	2.364	2.384	3.017**	1.571	1.642	1.505	1.811*	1.528
Relative weight (%)	9.14	9.34	9.75	10.27	15.10**	9.91	10.55	10.12	11.25*	10.27
Paleness	0/6	0/6	0/6	1/6	2/6	0/6	0/6	0/6	0/6	0/6
Foci	0/6	0/6	0/6	0/6	1/6	0/6	0/6	0/6	0/6	0/6
Tubular nephrosis	0/6	0/6	1/6	4/6	6/6	0/6	0/6	0/6	1/6	3/6
Liver:										
Absolute weight (g)	8.235	7.981	8.559	9.050	8.113	5.879	5.915	5.901	7.323**	6.853
Relative weight (%)	33.13	33.59	35.39	39.16**	40.10**	37.20	37.92	39.79	45.44*	46.23**

Courcy di Rosa (1988)
*$p < 0.05$, **$p < 0.01$

sumption, ophthalmology, haematology (including thrombocyte count, but not mean cell haemoglobin concentration or platelet count), serum biochemistry (except gamma glutamyl transferase), urine analysis (except microscopic examination), organ weights, gross pathology and histopathology (spleen, heart, adrenals, kidney, liver, lungs). No treatment-related mortality, ophthalmological changes or haematological alterations were observed and the only clinical sign noted was a blue discolouration of the tail in all animals at 1000 mg/kg bw per day; this was also noted at necropsy. Body-weight gains at this dose were 60% and 91% those of the controls for females and males respectively. Food consumption of the females at 1000 mg/kg bw per day was up to 20% less than that of the controls throughout the first 3 weeks of the study. Concentrations of glucose were reduced in males at 100 and 1000 mg/kg bw per day and in females at 1000 mg/kg bw per day. Concentrations of cholesterol were elevated in males at 1000 mg/kg bw per day and in females at 100 and 1000 mg/kg bw per day. Bilirubin and calcium concentrations were elevated in females at 1000 mg/kg bw per day. Significant increases in albumin and globulin concentrations and resultant total protein concentrations measured in males at 10 and 1000 mg/kg bw per day, were without a dose–response relationship, were small in magnitude and were consequently concluded to be incidental. The incidence of ketonuria and a yellow-green discolouration of the urine were noted in rats at 100 and 1000 mg/kg bw per day together with an increase in bilirubin concentration in females at 1000 mg/kg bw per day. The discolouration is likely to be due to the presence of a coloured metabolite. Occult blood in the urine was seen in two out of ten males at 1000 mg/kg bw per day. Liver weights relative to body weights were significantly increased at 1000 mg/kg bw per day in both sexes and at 100 mg/kg bw per day in females. The absolute weights of the liver and relative weights of the kidney were significantly increased at 100 and 1000 mg/kg bw per day in females. Females at 1000 mg/kg bw per day also had significantly elevated brain weights relative to body weights, secondary to reduced body-weight gains. Histopathological changes were confined to animals treated

at 1000 mg/kg bw per day and consisted of hepatocellular hypertrophy of the centrilobular region in two males and four females, pale casts in the renal tubules of two males, foreign material in the epidermis of the tail in two males and seven females, a fatty liver in one female, a fatty adrenal cortex in one female and lymphohistiocytic infiltration of the renal pelvis in one female. The NOAEL was 10 mg/kg bw per day on the basis of altered clinical chemistry at higher doses. The slight effects seen at 100 mg/kg bw per day suggest that this dose is a borderline NOAEL/lowest-observed-effect level (LOAEL) (Fankhauser, 1990).

Groups of 10 male and 10 female Sprague-Dawley rats were fed diets containing fludioxonil (purity, 96%) at a concentration of 0, 10, 100, 1000, 7000 or 20 000 ppm for 90 days. Actual achieved doses were 0, 0.8, 6.6, 64, 430, 1300 mg/kg bw per day for males and 0, 1.0, 7.1, 70, 460 or 1300 mg/kg bw per day for females. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, measurements of body weight and food consumption, ophthalmology, haematology (including erythrocyte volume fraction, clotting time, activated thromboplastin time, fibrinogen), serum biochemistry (including direct bilirubin, 5′nucleotidase and sorbitol dehydrogenase activity), urine analysis, organ weights, gross pathology and histopathology (including mammary glands and thigh muscle, but not gall bladder).

Rates of mortality were unaffected by treatment, although one male at 20 000 ppm died of unknown causes on day 36 and another male at 7000 ppm was terminated in a moribund condition with a pituitary adenoma on day 50. The excretion of a blue coloured metabolite caused the urine of animals at 1000, 7000 and 20 000 ppm to be coloured green-blue; at 7000 and 20 000 ppm, there was blue discolouration of the tail with discolouration of the abdomen and scrotum in the males, and blue or black discolouration of the tail and kidneys in nearly all rats and of the digestive tract in a few animals. Significantly lower body-weight gains were noted in females at 7000 ppm and in both sexes at 20 000 ppm, with a slight, non-significant, decrease in weight gains of males at 1000 ppm. Corresponding reductions in food intakes were noted. Ophthalmology in both sexes and haematology in males were unaffected. In females at 20 000 ppm, significant reductions were noted in the

Table 9. Clinical chemistry and organ weight changes in a 28-day study in rats treated with fludioxonil by gavage

Parameter	Dose (mg/kg bw per day)							
	Males				Females			
	0	10	100	1000	0	10	100	1000
Clinical chemistry								
Glucose (mmol/l)	7.866	7.875	6.794*	6.546*	6.576	6.968	6.074	5.459*
Bilirubin (µmol/l)	2.413	2.226	2.267	2.391	2.657	2.808	2.744	3.453*
Protein (g/l)	64.54	66.91	65.05	66.71*	66.75	66.38	64.63	65.78
Albumin (g/l)	36.05	37.34*	36.74	37.12*	37.71	37.48	36.68	37.50
Globulin (g/l)	28.49	29.57*	28.31	29.65*	29.04	28.90	27.95	28.28
Cholesterol (mmol/l)	1.640	1.757	1.638	2.040**	1.594	1.792	2.053*	2.171*
Calcium (mmol/l)	2.675	2.652	2.598	2.696	2.570	2.555	2.539	2.597*
Organ weight changes								
Liver, absolute (g)	18.14	17.93	19.09	18.78	10.55	11.30	12.04*	12.32*
Liver, relative to body weight	48.81	49.10	52.11	52.92*	42.40	45.59	48.33*	52.94**
Kidney, relative to body weight	6.943	6.871	7.002	7.438*	7.486	7.167	7.978*	8.003
Brain, relative to body weight	6.377	6.426	6.358	6.613	8.635	8.701	8.675	9.169*

From Fankhauser (1990)
* $p < 0.05$, ** $p < 0.01$

following parameters: concentration of haemoglobin (also noted at 7000 ppm), erythrocyte volume fraction, mean corpuscular volume and mean corpuscular haemoglobin. At 20 000 ppm, the following alterations in serum biochemistry were observed: decreased concentration of glucose, increased concentration of blood urea nitrogen, increased concentration of total bilirubin (also noted in males at 7000 ppm), increased cholesterol (also noted at 7000 ppm), and increased activity of gamma-glutamyl transferase. Females at 20 000 ppm also had significantly increased alkaline phosphatase activity and decreased 5′nucleotidase activity (also noted at 7000 ppm). An increase in the presence of bilirubin in urine was noted at 7000 and 20 000 ppm. The volume of urine was significantly decreased in females at 10, 7000 and 20 000 ppm. Given the absence of a significant effect at 100 and 1000 ppm, the effect at 10 ppm was concluded to be incidental.

Increased relative (to body) weights were observed at 7000 and 20 000 ppm for the adrenals (males only), brain, kidneys, liver, spleen and testes. The increase in relative weights of the brain and testes is likely to be secondary to reduced body weights, as the absolute weights of these organs tend to be conserved to some extent with reduced body-weight gains. Nonetheless, at 20 000 ppm absolute weights of the brain were decreased in females and absolute heart weights were decreased in both sexes. Absolute and relative weights of the thymus were significantly decreased at 7000 and 20 000 ppm. Five females and one male at 20 000 ppm were emaciated. Chronic nephropathy with active inflammation was noted in females at 20 000 ppm and in males at ≥1000 ppm. The incidence of chronic nephropathy was increased at 7000 ppm in males and at 20 000 ppm in both sexes. Hypertrophy of the centrilobular region of the liver was noted in one female at 100 ppm and in both sexes at ≥1000 ppm. Given the lack of statistical significance or a dose–response relationship in females at 100 and 1000 ppm, the single incidence at 10 ppm was discounted as incidental. As this is likely to reflect an adaptive response to the high xenobiotic load, it was also not considered to be an adverse effect at 1000 ppm, at which dose no evidence of liver damage from histology or clinical chemistry was observed. One male at 7000 ppm had a benign neoplasm in the pituitary and another had an adenoma of the pituitary. The NOAEL was 1000 ppm (64 mg/kg bw per day) on the basis of evidence of damage to the kidneys and liver at higher doses (Chang, 1990a).

Groups of five male and five female Tif:RAIf rats were dermally exposed to fludioxonil (purity, 97.5%) at a dose of at 0, 40, 200 or 1000 mg/kg bw per day, in 0.5% (w/v) carboxymethylcellulose in 0.1% (w/v) aqueous polysorbate 80, 5 days per week for 4 weeks. Fludioxonil was applied under an occlusive dressing that remained in place for 6 h and the application area was subsequently cleaned with lukewarm water. Control rats were exposed to a dressing containing the carrier under the same regime. Food, water and the housing environment were controlled and monitored. Toxicity was evaluated through: mortality, daily clinical observation, measurements of body weight and food consumption, skin irritation, haematology (including thrombocyte count, but not mean cell haemoglobin concentration or platelet count), serum biochemistry (except gamma-glutamyltransferase), organ weights, gross pathology and histopathology (skin at application site, skin at remote site, kidney, liver, thymus).

There were no deaths, no treatment-related clinical signs, no significant differences in body weights and, in males, no alteration in food consumption. For females at 1000 mg/kg bw per day, a slight increase in food intake was noted in the last 2 weeks. Organ weights, haematological and clinical biochemistry parameters were unaffected. Testicular atrophy was noted in one male at 1000 mg/kg bw per day, both macroscopically and microscopically.

Table 10. Principal observations in a 90-day study in rats fed diets containing fludioxonil

Parameter	Sex	Historical control range	Dietary concentration (ppm)					
			0	10	100	1000	7000	20 000
Haematology								
Haemoglobin (g/dl)	Males	14.5–17.3	15.3	15.7	15.5	15.6	15.5	14.9
	Females	13.5–16.9	15.6	15.7	15.9	15.2	14.7*	14.1**
Erythrocyte volume fraction (%)	Males	42.9–52.2	46.7	47.7	47.4	47.1	47.1	45.5
	Females	40.4–49.6	45.7	45.8	46.7	44.3	43.3	41.7**
Mean corpuscular volume (fl)	Males	46–57	54	54	54	53	53	53
	Females	53–60	55	55	55	54	54	52**
Mean corpuscular haemoglobin (per g)	Males	15.6–18.9	17.5	17.8	17.6	17.5	17.5	17.3
	Females	17.9–20.6	18.8	18.7	18.8	18.7	18.3	17.6**
Serum biochemistry								
Glucose (mg/dl)	Males	60–201	137	136	148	142	114	64**
	Females	49–183	123	98	121	93	84	63**
Blood urea nitrogen (mg/dl)	Males	12–22	16	15	16	15	15	20**
	Females	12–23	17	18	16	17	18	21*
Bilirubin (mg/dl)	Males	0.1–0.3	0.2	0.2	0.2	0.2	0.3*	0.4**
	Females	0.0–0.3	0.2	0.2	0.2	0.2	0.2	0.3*
Cholesterol (mg/dl)	Males	40–99	69	60	74	61	103*	109**
	Females	59–147	79	83	79	80	119*	122**
Gamma-glutamyl transferase (IU/l)	Males	4–6	5	5	4	4	5	10**
	Females	4–7	5	5	5	4	5	15**
Alkaline phosphatase (IU/l)	Males	68–145	91	92	98	111	92	110
	Females	20–119	54	54	64	54	59	97**
5′Nucleotidases (IU/l)	Males	11–43	16	13	16	13	12	11
	Females	11–25	21	24	21	18	14*	12**
Body and organ weights								
Body weight (g)	Males	—	577	601	591	546	518	418**
	Females	—	327	303	324	308	273**	227**
Adrenals, absolute (mg)	Males	—	57.5	59.9	65.6	57.6	69.9	63.9
	Females	—	59.9	62.1	84.3	64.0	60.0	55.1
Adrenals, relative to body weight	Males	—	0.010	0.010	0.011	0.011	0.0141*	0.0161**
	Females	—	0.020	0.022	0.027	0.023	0.0246	0.0283
Brain, absolute (g)	Males	—	2.145	2.273	2.160	2.195	2.245	2.090
	Females	—	1.966	1.980	1.962	1.965	1.934	1.858**
Brain, relative to body weight	Males	—	0.384	0.399	0.321	0.419	0.453**	0.541**
	Females	—	0.678	0.721	0.655	0.719	0.782*	0.950**
Heart, absolute (g)	Males	—	1.893	1.773	1.847	1.704	1.619	1.347**
	Females	—	1.074	1.110	1.125	1.053	1.029	0.816*
Heart, relative to brain weight	Males	—	88.25	78.10	85.61	77.70	72.660*	64.730**
	Females	—	54.66	56.02	57.36	53.67	52.320	43.913**
Kidneys, relative to body weight	Males	—	0.615	0.614	0.608	0.678	0.738*	0.857**
	Females	—	0.651	0.660	0.638	0.694	0.714	0.876**
Liver, relative to body weight	Males	—	2.734	2.644	2.817	2.827	3.323**	3.798**
	Females	—	2.596	2.624	2.603	2.791	3.667**	4.434**
Liver, relative to brain weight	Males	—	717.9	670.6	749.2	676.2	737.47	714.53
	Females	—	395.3	364.7	400.9	393.7	462.05	470.78*
Spleen, absolute (g)	Males	—	0.878	0.961	0.863	0.895	0.836	0.749
	Females	—	0.526	0.490	0.551	0.525	0.495	0.409**
Spleen, relative to body weight	Males	—	0.158	0.167	0.151	0.170	0.169	0.191**
	Females	—	0.171	0.178	0.184	0.191	0.202	0.210*
Thymus, absolute (g)	Males	—	0.339	0.315	0.366	0.358	0.227*	0.160**
	Females	—	0.226	0.216	0.263	0.263	0.205	0.124*
Thymus, relative to body weight	Males	—	0.059	0.054	0.064	0.067	0.046	0.040*
	Females	—	0.066	0.079	0.087	0.096*	0.083	0.063
Thymus, relative to brain weight	Males	—	15.86	13.72	16.94	16.28	10.191*	7.659*
	Females	—	11.41	11.00	13.35	13.34	10.508	6.663*
Testes, relative to body weight	Males	—	0.642	0.644	0.622	0.698	0.775*	0.944**
Histology								
Kidneys, inflammation	Males	—	0	0	0	1	6**	9**
	Females	—	0	0	0	0	0	9**
Kidneys, nephropathy	Males	—	3	3	2	4	10**	10**
	Females	—	0	0	0	0	0	10**
Kidneys, pelvis dilatation	Males	—	1	0	0	0	0	0
	Females	—	0	0	0	1	1	2
Liver, hypertrophy	Males	—	0	0	0	5	5	9**
	Females	—	0	0	1	1	6*	10**

From Chang (1990a)
$p < 0.05$, ** $p < 0.001$

One male from each of the grous at 40 and 200 mg/kg bw per day had mottled lungs that, after microscopic examination, were described as indicating chronic bronchopneumonia. Given the isolated occurence and lack of a dose–response relationship, these changes in the lung were concluded to be incidental to treatment. At 1000 mg/kg bw per day, all females displayed phagocytic cells in the thymus, one had lymphohistiocytic infiltration of remote skin and one had extramedullary haematopoiesis of the liver. Given the absence of such findings in studies in animals treated orally, the presence of phagocytic cells in the thymus is of uncertain relationship to treatment, but cannot be entirely discounted as incidental. Renal lymphohistiocytic infiltration was noted in one female from each of the groups at 200 and 1000 mg/kg bw per day. The NOAEL was 200 mg/kg bw per day on basis of the appearance of phagocytic cells in the thymus of females at 1000 mg/kg bw per day (Schneider, 1990).

Dogs

Groups of four male and four female beagle dogs (six males and six females in the control group and the group receiving a dose of 15 000/10 000 ppm) were fed diets containing fludioxonil (purity, 97.5%) at a concentration of 0, 200, 2000 or 15 000 ppm (0, 6.2, 59 and 290 mg/kg bw per day for males and 0, 6.2, 60 and 340 mg/kg bw per day for females) for 90 days. Owing to marked body-weight loss and reduced food consumption at 15 000 ppm in the first 3 weeks, the dose was reduced to 10 000 ppm. The highest dose is referred to hereafter as 10 000 ppm. At the end of the 90-day period of treatment, two dogs of each sex in the control group and at 10 000 ppm were allowed to recover on control diets for 4 weeks. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, measurements of body weight and food consumption, ophthalmology, haematology (including erythrocyte volume fraction and thrombocyte count, but not platelet count or prothrombin), serum biochemistry, urine analysis (including nitrites), organ weights (including pituitary, thyroid, parathyroid, but not thymus), gross pathology and histopathology (including mammary glands, but not gall bladder).

No deaths occurred during the study and ophthalmology and gross pathology were normal. Green discolouration of the digestive tract was noted in one male at 2000 ppm and three dogs of each sex at 10 000 ppm. Blue faeces were noted in dogs at 2000 and 10 000 ppm. This discolouration was attributed to a coloured metabolite and was not considered to be toxicologically relevant. Three dogs of each sex at 2000 ppm and all dogs at 10 000 ppm had intermittent diarrhoea. Within 2 days of cessation of treatment, faeces were normal for all animals. All other effects reported below occurred at 10 000 ppm only. Body weights of both sexes were reduced and food intake was markedly reduced, by up to 50%, during the first 3 weeks; after the dose was reduced no significant variations were noted. Erythrocyte counts were lower for both sexes at week 13 and recovered only partially on cessation of treatment. Reduced concentrations of haemoglobin were also noted at weeks 8 and 13, but not week 4. Mean cell haemoglobin concentration was reduced and thrombocyte count and fibrinogen concentrations were elevated, and the mean corpuscular volume was elevated in females; however, these effects were reversible. A significant increase in serum cholesterol concentrations was noted in females, but was reversible. Liver weight, both absolute and relative to body weight, was increased in both sexes and may have been related to increased severity, but not incidence, of bile duct proliferation, found in all groups at this dose. The NOAEL was 2000 ppm (59 mg/kg bw per day) on the basis of anaemia and increased serum cholesterol concentrations at 10 000 ppm. As the intermittent diarrhoea did

Table 11. Key findings of a 90-day study in dogs fed diets containing fludioxonil

Parameter	Dietary concentration (ppm)							
	Male				Female			
	0	200	2000	10 000	0	200	2000	10 000
Erythrocyte count (10^6/mm^3)	6.89	6.95	6.45	6.48	6.53	6.79	6.6	5.46*
Haemoglobin (g/dl)	16.2	16.7	15.2	15.2	15.4	16.1	15.8	12.9*
Fibrin (g/l)	1.93	1.53	1.63	2.12	1.72	1.42	1.4	2.28**
Platelets (g/l)	310	325	337	440**	397	336	432	527*
Cholesterol (mmol/l)	4.2	4.6	4.2	4.7	4.4	3.3	3.7	6.9**

From Moysan (1990)
$p < 0.05$, ** $p < 0.001$

not affect food consumption or the condition of the animals, was readily reversible and was not observed in a 1-year study at doses of up to 8000 ppm, it was considered unlikely to reflect systemic toxicity and was not therefore considered in relation to the the NOAEL (Moysan, 1990).

Groups of four male and four female beagle dogs were fed diets containing fludioxonil (purity, 97.5%) at a concentration of 0, 100, 1000 or 8000 ppm (0, 3.1, 33 or 300 mg/kg bw per day in males and 0, 3.3, 36 or 330 mg/kg bw per day in females) for 52 weeks. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, measurements of body weight and food consumption, ophthalmology, haematology (including thrombocyte count, fibrinogen, activated thromboplastin time, erythrocyte volume fraction and quick time), serum biochemistry (including creatine kinase), urine analysis (including nitrites), organ weights (including pituitary, thyroids and parathyroids, but not thymus), gross pathology and histopathology (including mammary glands).

There were no deaths and food consumption, urine analysis parameters and ophthalmology were unaffected. Blue faeces were noted in all dogs at 1000 and 8000 mg/kg and those at 8000 ppm had discolouration of the digestive tract. Animals receiving fludioxonil at a dietary concentration of 8000 ppm weighed considerably less than control animals throughout the study. A single female at 1000 ppm lost weight from week 22 to week 32 and intermittently subsequently, and finished the study with a lower body weight than any animal at 8000 ppm. The remaining animals at this dose had body weights comparable to those of the controls. Given the pattern of weight change in this individual animal, it was concluded to be incidental to treatment. One male at 8000 ppm displayed alopecia from week 44. In males at 8000 mg/kg, the thrombocyte count and fibrinogen concentrations were consistently elevated throughout the study, and prothrombin time was reduced at week 52. These values were all within the ranges for historical controls. Platelet counts were increased significantly in males and non-significantly in females at 8000 ppm. Although the haematology changes are small in magnitude, they are generally consistent with those seen in the 90-day study, including slight non-significant decreases in erythrocyte and haemoglobin values, and it was therefore concluded that they were related to treatment. Significant increases in serum cholesterol concentrations, above the range for historical controls, were noted in males at 8000 ppm and total bilirubin concentrations were slightly elevated in all dogs at 8000 ppm throughout the study. At 8000 ppm, increased liver weights (correlating with visibly enlarged livers in two females) and decreased spleen weights were noted in both sexes and heart weights in males were reduced. Two females at 8000 ppm had enlarged

Table 12. Key findings of a 12-month study in dogs fed diets containing fludioxonil

Parameter	Sex	Dietary concentration (ppm)			
		0	100	1000	8000
Cholesterol (mmol/l)	Male	2.8	3.2	3.0	4.7*
	Female	4.8	4.1	4.0	4.8
Total bilirubin (µmol/l)	Male	2	2	1	3
	Female	2	2	2	4
Prothrombin time (s)	Male	7.0	6.6	6.8	6.3*
	Female	6.5	6.3	6.6	6.4
Fibrin (g/l)	Male	1.87	2.27	2.02	2.78*
	Female	2.04	1.89	2.06	2.11
Platelets (10^3/mm^3)	Male	316	324	368	413*
	Female	402	395	442	500
Body and organ weights					
Body weight (kg)	Male	11.5	12.4	12.5	9.7
	Female	12.3	12.0	11.1	10.1
Liver	Male	288.2	265.5	297.0	305.6
	Female	285.7	266.3	267.8	324.9*
Spleen	Male	25.03	24.39	25.20	18.07
	Female	29.24	21.26	24.33	24.08
Heart	Male	95.87	91.87	96.95	72.37**
	Female	85.46	83.09	84.47	81.30

From Vallet (1992)
$p < 0.05$, ** $p < 0.001$

livers. The size of the spleen was reduced in two males and one female at 8000 ppm and in one male at 100 ppm. The size of the thymus was reduced in two dogs of each sex at 8000 ppm and in one male at 1000 ppm. Other than biliary epithelial cell proliferation observed in one female at 8000 ppm, no histological abnormalities were noted. The NOAEL was 1000 ppm (33 mg/kg bw per day) on the basis of reduced body-weight gains, increased cholesterol concentrations and altered haematology at 8000 ppm. Given the mild degree of the effects observed at 8000 ppm and the wide dose spacing between the NOAEL and LOAEL, the Meeting concluded that the NOAEL is conservative (Vallet, 1992).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 60 male and 60 female CD-1 mice were fed diets containing fludioxonil (purity, 95.4%) at a concentration of 0, 10, 100, 1000 or 3000 ppm (0, 1.1, 11.3, 112 or 360 mg/kg bw per day for males and 0, 1.4, 16.5, 133 or 417 mg/kg bw per day for females) for 18 months. Ten mice of each sex per group were terminated after 12 months. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, body weight, measurements of food and water consumption, ophthalmology, haematology (including reticulocyte time), organ weights, gross pathology and histopathology. The survival patterns for control and treated groups were similar. Pelvic staining, blue discolouration of the skin, stomach and urine seen in some animals at 1000 and 3000 ppm were attributed to a coloured metabolite in the urine and discounted as toxicologically irrelevant. A slight decrease (statistically significant from week 10 to 78) in body-weight gain was noted in males at 3000 ppm; at various stages throughout the study these males consumed significantly more food than did the controls. Water consumption, only measured for weeks 50 and 51, was increased for males at 3000 ppm.

Haematology and ophthalmology were unaffected by treatment, with the exception of females at 1000 and 3000 for males at 3000 ppm for whom a slightly, but significantly, reduced mean corpuscular haemoglobin concentration (controls, 34.52%; 1000 ppm, 33.6%**; 3000 ppm, 33.6**; where ** $p < 0.01$) was observed at 18 months. Because of the small magnitude of the effect in this study, the absence of correlating haematology findings and the lack of a dose–response relationship, it was concluded that the reduced mean cell haemoglobin concentration, in isolation, was of equivocal relationship to treatment and was not considered in the identification of a NOAEL for this study, despite the statistical significance and the fact that it was consistent with weak anaemia at 7000 ppm in a second study in mice. At 3000 ppm, liver weights, absolute and/or relative, were elevated. The incidences of enlarged spleen (males and females), liver (females), thymus (females) and lymph nodes (males and females) were increased at 3000 ppm. There were no significant variations noted in the incidence of non-neoplastic lesions. There was a significant increase in the incidence of lymphoma in females at 3000 ppm (11, 10, 13, 12, 18 for the control group to the group receiving a dose of 3000 ppm, respectively). Lymphoma is a common finding in ageing female CD-1 mice and the historical incidence at the conducting laboratory is 13–32%, which is comparable to the incidence noted in females at 3000 ppm. Lymphoma was the cause of death of more females at 3000 ppm than in any other group (1, 0, 2, 4, 6 for the control group to the group receiving a dose of 3000 ppm, respectively). Given the high background rate of this finding and the absence of any increase in another study by the same authors using higher doses, the apparent increase in lymphomas observed in this study was concluded to be incidental. No significant variations in the incidence of neoplastic lesions were noted. The NOAEL was 1000 ppm (112 mg/kg bw per day) on the basis of increased liver weights, and thymic and splenic enlargement at 3000 ppm (Chang & Wyand, 1993a).

Groups of 60 male and 60 female CD-1 mice were fed diets containing fludioxonil (purity, 95.4%) at a concentration of 0, 3, 30, 5000 or 7000 ppm (0, 0.33, 3.3, 590 or 850 mg/kg bw per day for males, and 0, 0.41, 4.1, 715 or 1000 mg/kg bw per day for females) for 18 months. Ten mice of each sex per group were terminated after 12 months. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in

Table 13. Key findings of an 18-month study in mice given diets containing fludioxonil

Parameter	Sex	Dietary concentration (ppm)				
		0	10	100	1000	3000
Body weight	Male	40.8	42.3	43.1	42.0	40.0
	Female	32.0	34.8	34.3	34.5	33.4
Liver weight, absolute	Male	1.674	1.663	1.728	1.763	1.822
	Female	1.382	1.454	1.487	1.476	1.612**
Liver weight, relative to body weight	Male	4.168	4.031	4.084	4.183	4.518
	Female	4.366	4.262	4.441	4.282	4.887*
Liver weight, relative to brain weight	Male	325.151	324.139	330.177	338.419	361.290*
	Female	272.003	285.332	292.107	286.882	317.480**
Enlarged spleen	Male	14	16	9	18	22
	Female	19	22	21	26	28
Enlarged liver	Male	1	2	2	2	3
	Female	1	2	1	3	6
Enlarged thymus	Male	3	0	1	4	0
	Female	4	8	7	10	13
Enlarged lymph nodes	Male	1	2	3	3	6
	Female	0	3	4	3	6

From Chang & Wyand (1993a)
$p < 0.05$, ** $p < 0.001$

the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, measurements of body weight and food consumption, ophthalmology, haematology (including reticulocyte time), organ weights, gross pathology and histopathology.

At 7000 ppm, survival was markedly decreased (control: males, 76% and females, 71%; 7000 ppm: males, 27% and females, 22%) and animals displayed rough coat, hypothermia, reduced activity, pallor, distended abdomen, loose stools, dehydration, dyspnoea, discoloured stools, stained anus, discoloured urine and stained pelvic region. At 5000 ppm, rough coat, pallor and loose stools, were observed. Weight gains in males were 8% and 25% below those of controls for males at 5000 and 7000 ppm and 20% below those of controls for females at these doses. Discolouration of the urine, anus, pelvic region and some internal organs seen at 5000 and 7000 ppm were attributed to a coloured metabolite of fludioxonil and discounted as toxicologically irrelevant. Slight anaemia was observed in both sexes at 7000 ppm from 12 months. At 7000 ppm, absolute and relative weights of the kidney were significantly reduced in males (also at 5000 ppm) and elevated in females. Despite the unusual pattern of this change in the two sexes, as it correlates with nephropathy accompanied by calcification of renal tubules at 5000 and 7000 ppm, which was implicated in the death of 31 males and 32 females at 7000 ppm, it was considered to be a reflection of treatment. Absolute and relative weights of the liver in both sexes were elevated at 5000 and 7000 ppm. Rough coats, an increased incidence of liver cysts, renal pitting and renal cysts were seen in both sexes at 7000 ppm, and an elevated incidence of liver necrosis and bile duct hyperplasia was seen in males and a slight increase in the incidence of haemorrhage of the lungs was seen in females at this dose. There was no increase in the number or type of neoplastic lesions. The incidence of lymphomas, which appeared to be increased in the previous study, in females was similar to that in controls (control, 11; 3 ppm, 7; 30 ppm, 12; 5000 ppm, 11; 7000 ppm, 8). The NOAEL was 30 ppm (3.3 mg/kg bw per day) on the basis of decreased body-weight gains, increased liver weights and nephropathy at the next higher dose (Chang & Wyand, 1993b).

The two 18-month studies used the same strain and source of mice, the same batch of fludioxonil and were conducted in the same laboratory with only a difference of 6 months between the commencement of the first and second study. The overall NOAEL for these two studies was 1000 ppm (112 mg/kg bw per day).

Rats

Groups of 50 male and 50 female Sprague-Dawley rats were fed diets containing fludioxonil (purity, 95.4%) at a concentration of 0, 10, 30, 100, 1000 or 3000 ppm (0, 0.37, 1.1, 3.7, 37 or 110 mg/kg bw per day for males, and 0, 0.44, 1.3, 4.4, 44 or 140 mg/kg bw per day) for up to 24 months. Twenty rats of each sex per group were maintained for clinical laboratory tests. An additional ten rats of each sex per group were terminated after 12 months, and a further ten rats of each sex from the control group and the group at 3000 ppm were treated for 12 months, removed from the test diet for 1 month and then terminated. A further nine males from each group were maintained for studies of metabolism. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observation, measurements of body weight, food and water consumption, ophthalmology, haematology, serum biochemistry (including creatine kinase, 5-nucleotidase, sorbitol dehydrogenase), organ weights (except thymus), gross pathology and histopathology (including cervix).

Table 14. Key observations in an 18-month study in mice given diets containing fludioxonil

Parameter	Sex	Dietary concentration (ppm)				
		0	3	30	5000	7000
Haematology						
Erythrocytes (10^6/µl)	Male	8.83	8.72	8.34	8.00	7.27
	Female	8.39	8.60	8.74	8.32	5.90**
Haemoglobin (g/dl)	Male	13.6	13.1	12.7	11.9	10.9*
	Female	12.7	12.4	13.2	12.5	8.9**
Erythrocyte volume fraction (%)	Male	37.9	36.8	35.2	33.1	30.5*
	Female	36.4	35.4	37.6	35.4	26.0**
Reticulocytes (%)	Male	2.1	2.3	2.0	2.9	3.4
	Female	1.8	1.9	1.4	2.5	2.7
Body and organ weights						
Body weights	Male	42	42.5	42.6	41.2	38.2*
	Female	37.7	36.0	35.9	34.5**	35.0
Kidneys, absolute (g)	Male	0.664	0.667	0.680	0.597*	0.527**
	Female	0.429	0.450	0.441	0.428	0.497*
Kidneys, relative to body weight	Male	1.763	1.724	1.774	1.628	1.524**
	Female	1.280	1.391	1.354	1.356	1.570**
Liver, absolute (g)	Male	1.583	1.588	1.631	1.789*	1.696
	Female	1.385	1.481	1.376	1.652**	1.852**
Liver, relative to body weight	Male	4.253	4.186	4.336	4.843*	4.978
	Female	4.119	4.562	4.221	5.240**	5.798**
Gross pathology						
Rough coat	Male	1	4	6	5	27
	Female	1	2	4	4	21
Liver cysts	Male	0	2	1	2	8
	Female	1	1	0	0	1
Kidneys, rough pitted surface	Male	0	2	1	4	29
	Female	1	0	1	5	21
Kidney cysts	Male	8	18	11	9	17
	Female	8	3	5	10	17
Histopathology						
Nephropathy	Male	0	0	0	53	59
	Female	0	0	0	21	58
Liver necrosis	Male	2	3	4	7	13
	Female	5	5	3	1	5
Bile duct, hyperplasia	Male	0	0	1	4	23
Kidneys, calcification	Male	1	5	5	13	45
	Female	2	2	1	18	56
Lungs, haemorrhage	Male	1	1	2	2	1
	Female	0	1	2	3	8

From Chang & Wyand (1993b)
$p < 0.05$, ** $p < 0.001$

Treatment did not affect survival rates, ophthalmology, food and water consumption or clinical signs, other than the observation of some diarrhoea at 3000 ppm. Blue staining of the urine, stools and fur were attributed to a coloured metabolite and discounted as toxicologically irrelevant. Final body weights were lower in both sexes at 3000 ppm, although this did not reach statistical significance. Body-weight gains were significantly reduced at 3000 ppm (by 11%) in both sexes. Slight anaemia and slight decreases in leukocyte counts were noted at 3000 ppm. For males, statistical variations in bilirubin, creatine, gamma-glutamyl transferase and creatinine kinase activities were noted at 6 months, however these trends were not noted at 12, 18 and 24 months. In treated females, gamma-glutamyl transferase activities were statistically, but not toxicologically, significantly increased at 6, 18 and 24 months. The values were generally quite low in absolute terms and comparable to the range for historical controls. Although absolute organ weights were comparable to those of the controls, slightly elevated weights of kidneys and livers relative to body weight were noted in males at 3000 ppm. Increased relative weight of the testes at 24 months reflected

lower body weight and is not toxicologically significant. Males at 3000 ppm had increased incidences of enlarged liver, hepatocellular hypertrophy, nephropathy and renal cysts (also observed at 1000 ppm, but not confirmed histologically). Histologically, the incidence of renal cysts was increased in males at 3000 ppm only. Increased incidences of centrilobular necrosis and basophilic foci of the liver were noted at 3000 ppm in both sexes. An increase in the number of hepatocellular tumours (benign plus malignant) were also noted in females at 3000 ppm, but the incidence was within the historical range (adenoma, 0–10%; carcinoma, 0–1.7%; adenoma plus carcinoma, 0–10%), there were no histological changes indicative of a proliferative response (e.g. foci of cellular alteration) and the tumors were discrete nodules. The overall incidence of neoplasms and the number of animals with neoplasms were similar for control and treated groups. The NOAEL was 1000 ppm (37 mg/kg bw per day) on the basis of an increased incidence of renal cysts and nephrosis in males and decreased body-weight gains in both sexes at the next higher dose (Chang & Richter, 1993).

A subsequent re-examination of the slides of livers from this study (Emeigh Hart, 1994) was performed in order to determine whether the slight increase in hepatocellular tumours in females at 3000 ppm was attributable to a compound-related mitogenic effect and, if so, to identify a no-observed-effect level (NOEL) for this effect. An immunohistochemical method was used to detect proliferating cell nuclear antigen (PCNA). A slight, dose-related but not statistically significant, increase in the labelling index was noted in females at 12 months (labelling index: 0.35, 0.37, 0.49, 0.67, 0.56 for dietary concentrations from 0 to 3000 ppm respectively), but not at 13 months (i.e. after fludioxonil had been withdrawn for 1 month) indicating rapid reversibility of cell proliferation on compound withdrawal. Males were unaffected. The overall NOAEL of 1000 ppm was confirmed.

2.4 Genotoxicity

The results of studies of genotoxicity with fludioxonil are summarized in Table 16.

2.5 Reproductive toxicity: multigeneration studies

Rats

In a two-generation study, groups of 30 Sprague-Dawley rats were given diets containing fludioxonil (purity, 95.4%) at a concentration of 0, 30, 300 or 3000 ppm. Actual intakes of fludioxonil were calculated (weighted average) as 0, 2.2, 22 or 230 mg/kg bw per day for males and 0, 2.0, 21 or 210 mg/kg bw per day for females, for the control group to the group receiving 3000 ppm respectively. Treatment of the parental (F_0) rats commenced 70 days before cohabitation, continued through cohabitation (up to 21 days) and was subsequently continued for females until pups were weaned at day 21 of lactation. Parental males and females, and unmated females were terminated after weaning of the pups. Pups from the F_0 generation were culled to four males and four females on postnatal day 4 where possible. At postnatal day 21, pups of the F_0 dams (one male and one female from each litter where possible) were randomly selected for the F_1 generation and the remainder were terminated. Food, water and the housing environment were controlled and monitored. The amount of fludioxonil in the diet was analysed regularly. Toxicity was evaluated through: mortality, daily clinical observations, measurements of body weight and food consumption, gross pathology, litter parameters, mating performance, fertility indices, reproduction parameters, survival indices, sex ratio, organ weights (testes and ovaries only) and histopathology (uterus and gross lesions).

Table 15. Key findings of a 2-year study in rats given diets containing fludioxonil

Parameter	Sex	Time-point (months)	Dietary concentration (ppm)					
			0	10	30	100	1000	3000
Haematology								
Leukocytes (10^3/µl)	Male	12	13.6	11.8	11.1	12.3	11.9	10.9
		24	12.2	13.0	13.1	14.3	13.0	10.4
	Female	12	7.1	6.6	6.6	7.3	6.1	6.6
		24	9.4	9.0	8.4	10.2	9.5	8.5
Erythrocytes (10^6/µl)	Male	12	9.14	9.26	9.07	9.25	8.98	9.04
		24	8.34	8.11	8.27	8.21	8.12	8.04
	Female	12	8.32	8.46	8.43	8.28	8.46	7.91*
		24	7.54	7.13	7.57	6.95	6.77	6.72
Haemoglobin (g/dl)	Male	12	15.9	16.0	16.1	16.0	15.7	15.7
		24	14.7	14.4	14.6	14.3	14.4	14.1
	Female	12	15.3	15.6	15.6	15.2	15.4	14.4**
		24	14.0	13.5	14.3	12.9	13.0	12.5
Erythrocyte volume fraction (%)	Male	12	47.5	17.9	47.6	47.8	47.4	48.0
		24	43.6	42.9	43.1	42.8	43.1	42.3
	Female	12	45.4	46.2	46.5	45.5	45.9	45.1
		24	41.6	39.7	41.8	38.2	38.2	37.1
Serum biochemistry								(Historical controls)
Gamma-glutamyl transferase (µg/l)	Male	18	5	7*	7*	5	7*	7* (0–20)
		24	3	6**	6**	5	6**	7** (1–7)
	Female	18	2	3	4**	4**	4**	4** (0–8)
		24	5	7*	7*	7*	7*	7* (0–6)
Body and organ weights								
Body weight (g)	Male	24	733	791	794	828	757	697
	Female	24	564	595	561	552	583	518
Liver, relative	Male	12	2.383	2.371	2.429	2.329	2.497	2.704*
		24	2.614	2.625	2.756	2.443	2.658	2.868
	Female	12	2.423	2.334	2.528	2.412	2.632	2.507
		24	3.083	4.643	3.193	3.345	4.023	4.387
Kidneys, relative	Male	12	0.551	0.545	0.538	0.552	0.518	0.547
		24	0.766	0.679	0.716	0.680	0.727	0.806
	Female	12	0.580	0.510	0.570	0.567	0.637	0.559
		24	0.557	0.563	0.530	0.615	0.524	0.577
Testes, relative	Male	12	0.525	0.499	0.526	0.499	0.519	0.502
		24	0.473	0.478	0.487	0.434	0.479	0.557*
Gross pathology	*n*		70	60	60	60	60	70
Kidney cysts	Male	24	6	7	4	9	12	11
	Female	24	0	1	4	2	1	3
Histology	*n*		70	60	60	60	60	70
Enlarged liver	Male	24	2	4	3	3	3	12
	Female	24	6	3	1	4	0	3
Liver, degeneration/atrophy/ centrilobular necrosis	Male	24	4	8	4	5	9	16
	Female	24	8	5	9	9	13	21
Liver, hepatocellular hypertrophy	Male	24	17	16	15	21	16	27
	Female	24	19	13	4	5	13	15
Hepatocellular carcinoma[a]	Female	24	0	0	0	0	0	1
Hepatocellular adenoma	Female	24	0	2	0	0	0	4
Kidney cysts	Male	24	18	6	5	7	11	28
	Female	24	0	1	2	1	0	3
Nephrosis	Male	24	59	45	47	49	51	67

From Chang & Richter (1993)

[a] In males, the historical incidence of hepatocellular tumours (adenomas plus carcinomas) ranges from 1.4% to 15%. In females the historical incidence of hepatocellular tumours ranges from 0% to 10%

$p < 0.05$, ** $p < 0.001$

Table 16. Results of studies of genotoxicity with fludioxonil

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium*, plate incorporation	0, 200, 780, 3130, 12 500 or 50 000 µg/ml ±S9, in acetone	NS	Negative	Ogorek (1987)[a]
Reverse mutation	*S. typhimurium*, broth culture	0, 0.976, 3.91, 16.5, 250 or 1000 µg/ml ±S9, in DMSO	NS	Negative	Deparade (1987)
Reverse mutation	*S. typhimurium*, *E. coli*, ±S9, plate incorporation	0, 200, 780, 3130, 12 500 or 50 000 µg/ml ±S9, in acetone	97.5	Negative	Ogorek (1989)
Gene mutation	Chinese hamster V79 cells	0, 0.5, 1.0, 2.0, 4.0, 8.0, 10.0, 12.0, 16.0 or 20.0 µg/ml −S9, in DMSO	97.5	Negative	Dollenmeier (1989)
		0, 1.5, 3.0, 6.0, 12.0, 18.0, 24.0, 30.0, 36.6, 48.0 or 60.0 µg/ml +S9, in DMSO		Negative	
Chromosomal aberration	Chinese hamster ovary CCL 61 cells	0, 10.94, 21.88 or 43.75 µg/ml (3 h) −S9, in DMSO	97.5	Positive	Strasser (1989)
		0, 2.73, 5.47 or 10.94 µg/ml (24 h) −S9, in DMSO		Negative	
		0, 5.74, 10.94, 21.88, 43.75, 87.5, 175.0 or 350.0 µg/ml +S9, in DMSO		Weak Positive[a]	
Unscheduled DNA synthesis	Rat hepatocytes	0, 4.1, 12.3, 37, 111, 333, 1000, 2500 or 5000 µg/ml	97.5	Negative	Hertner (1989)
In vivo					
Micronucleus formation	Tif:MAGf mice	0, 1250, 2500 or 5000 mg/kg bw in aqueous carboxy methyl cellulose	97.5	Negative	Hertner (1990)
Micronucleus formation	Tif:RAIf rats	0, 1250, 2500, 5000 mg/kg bw in aqueous carboxy methyl cellulose	97.5	Negative	Meyer (1991), Ogorek (1999)
Chromosomal aberration	Chinese hamster	0, 1250, 2500 or 5000 mg/kg bw in aqueous carboxy methyl cellulose	96.4	Negative	Hertner (1993a)
Dominant lethal mutation	Tif:MAGf mice	0, 1250, 2500 or 5000 mg/kg bw in aqueous carboxy methyl cellulose	96.4	Negative	Hertner (1992a)
DNA repair synthesis	Tif:RAIf rats	0, 2500 or 5000 mg/kg bw in aqueous carboxy methyl cellulose	96.4	Negative	Hertner (1993c)
Replicative DNA synthesis				Negative	
Bone marrow aneuploidy	Crl:CD(SD)BR rats	1250, 2500 or 5000 mg/kg bw in aqueous carboxy methyl cellulose	97.5	Negative	Myhr (1999)
Dominant lethal mutation	Tif:MAGf mice	1250, 2500, 5000 mg/kg bw per day	96.4	Negative	Hertner (1992b)

DMSO, dimethyl sulfoxide; NS, not stated

[a] GLP status unknown

[b] Less than a doubling of the control value, but statistically significantly higher (aberration rates: negative control, 6%; positive control, 84%; 87.5 and 175 µg/ml, 11%) at non-cytotoxic concentrations

Parental F_0 males at 3000 ppm ate, and weighed (<5% reduction in weight gains), slightly less than the controls, and F_1 males had slightly but significantly reduced food consumption and weight gains (<10%). Weight gains in F_0 parental females at 3000 ppm were generally lower than those of controls, reaching significance at some time-points, but were largely unaffected in F_1 females. Food consumption was reduced in the F_0 but not F_1 females. Absolute weights of the testes were similar in the control and treated groups, however, F_1 males at 3000 ppm had significantly higher testicular weights relative to body weights (control, −0.63; 3000 ppm, −0.68, $p < 0.05$) reflecting the lower body weights (testes weights tend to be conserved where weight gain is reduced). Treatment did not affect the number of females mating within 21 days, fertility or reproduction parameters, or the length of gestation. The number of implantation sites per dam was significantly higher for F_1 groups at 300 and 3000 ppm compared with controls, which is clearly not an adverse effect and is likely to be an incidental finding. Absolute weights of the ovary were significantly lower in F_0 females at 30 and 3000 ppm than in controls, although no dose-related response was noted (control, −0.12 g; 30 ppm, −0.10* g; 300 ppm, −0.11 g; 3000 ppm, −0.10* g; where

* is $p < 0.05$) and no effect was seen in F_1 females. It was consequently concluded that this finding was incidental. No significant differences in survival indices were noted. One F_0 dam treated with fludioxonil at 3000 ppm had 13 pups with no milk in their stomachs. F_1 and F_2 pups at 3000 ppm weighed significantly less than controls (8–12% by day 14 and similar difference at day 21). This effect is not attributable to increased litter size or effects on parental weight gains as these were not consistent across the two generations of pups. The effects are also not attributable to consumption of maternal diet by the pups as the extent of the effect was maximal on day 14 with no progression to day 21. Consumption of maternal diet increased substantially between days 14 and 21. No treatment-related gross pathological changes were noted in culled pups and weanlings. The mean litter size was significantly increased in F_2 pups at 300 and 3000 ppm; this is probably an incidental finding and is clearly not adverse. The mean proportion of pups surviving to day 4 was significantly decreased for the F_2 generation at 3000 ppm, but was similar to the F_1 control value for this parameter. Also the number of pup deaths in the control group on postnatal days 0–21 was 3.9 per litter for F_1 pups and 1.3 per litter for F_2 pups, compared with 3.1 per litter and 4.2 per litter for the F_1 and F_2 pups at 3000 ppm, respectively, suggesting that the apparent decrease in survival rate in the F_2 pups at 3000 ppm was artifactual. The NOAEL for parental toxicity was 300 ppm (21 mg/kg bw per day) on the basis of slightly lower body weights and food intake in males at the next higher dose. The NOAEL for pup development was 300 ppm (21 mg/kg bw per day) on the basis of lower body weights at the next higher dose. There were no effects on the reproductive success of parental animals at the highest dose (Singh, 1992).

Sprague-Dawley rats were mated, with the day on which a sperm-positive vaginal smear was obtained being designated day 0. Groups of 25 successfully mated females were then treated with fludioxonil (purity, 97.5%) at a dose of 0, 10, 100 or 1000 mg/kg bw per day by gavage on days 6–15 of gestation. Maternal toxicity was evaluated using: rate of mortality, daily clinical observations, measurements of body weight and food consumption, and dissection and examination of the reproductive tract. Fetuses were removed by caesarean section on day 20 of gestation, and toxicity was determined by external, visceral and skeletal examination, and measurement of body weight. Food, water and the housing environment were controlled and monitored. One female in the control group died during the study and two dams in the groups receiving a dose of 10 or 100 mg/kg bw per day had total resorptions. No treatment-related clinical signs were noted. Females treated with fludiox-

Table 17. Body weights (g/% of that of controls) of pups in a two-generation study of reproductive toxicity in rats fed diets containing fludioxonil

Generation	Postnatal day	Dietary concentration (ppm)							
		Males				Females			
		0	30	300	3000	0	30	300	3000
F_1	0	6.64/100	6.52/98	6.64/100	6.40/96	6.19/100	6.16/100	6.30/102	5.99/97
	4[a]	10.18/100	9.88/97	10.47/103	9.28*/91	9.59/100	9.40/98	10.02/104	8.80/92
	14	33.60/100	31.68/94	33.62/100	29.72**/88	32.17/100	30.23/94	32.39/101	28.35**/88
	21	56.87/100	54.34/96	56.68/100	50.27**/88	53.73/100	51.55/96	54.28/101	47.62**/89
F_2	0	6.38/100	6.33/99	6.21/97	6.12/96	6.07/100	5.90/97	5.88/97	5.78/95
	4[a]	9.15/100	9.64/105	9.03/99	8.67/95	8.89/100	8.96/101	8.76/99	8.16/92
	14	30.05/100	30.74/102	30.93/103	27.97*/93	29.59/100	29.49/100	30.17/102	26.12**/88
	21	48.67/100	50.53/104	51.07/105	44.62*/92	47.51/100	47.88/101	49.43/104	41.69**/88

From Singh (1992)

[a] Weight of remaining pups after culling

*$p < 0.5$, **$p < 0.01$

Table 18. Litter parameters in a two-generation study of reproductive toxicity in rats fed diets containing fludioxonil

Parameter	Dietary concentration (ppm)			
	0	30	300	3000
F_1 generation				
No. of implantation sites	16.2	14.8	15.0	15.3
Mean litter size (day 0)	15.0	13.3	13.6	14.7
Mean % of pups that survived until day 4	96.9	98.5	98.3	98.3
F_2 generation				
No. of implantation sites	14.86	12.68	16.42*	16.32*
Mean litter size (day 0)	14.1	12.2	15.1*	15.5*
Mean % of pups that survived until day 4	98.8	93.7	98.4	96.3*

From Singh (1992)
*$p < 0.5$, **$p < 0.01$

Table 19. Findings in fetuses in a study of developmental toxicity in rats given fludioxonil by gavage

Parameter	Dose (mg/kg bw per day)			
	0	10	100	1000
Body weight (g)	3.73 ± 0.39	3.86 ± 0.50	3.91 ± 0.45	3.95 ± 0.41
Dilated renal pelvis	1 (0.8%)	1	1	5 (4.0%)
Dilated ureter	4 (3.1%)	2	5	7 (5.6%)

From Savary (1989a)

onil at 1000 mg/kg bw per day had significantly lower body-weight gains during gestation (control, −98.3 g; 1000 mg/kg bw per day, −82.2 g). A slight decrease in food consumption at 1000 mg/kg bw per day was noted from day 6 to day 11 of gestation. Treatment had no effect on the number of live implants per dam, rate of resorptions, pup sex ratio, or fetal external or skeletal alterations. There was a very slight, non-significant increase in pup weight with dose. Slight increases in the incidence of dilated renal pelvis and dilated ureter were noted in fetuses at 1000 mg/kg bw per day, but remained well within the range for historical controls (0.6–7.5%) and the pattern of the dose–response relationship suggests that this is an incidental finding. The NOAEL for fetal development was 1000 mg/kg bw per day, the highest dose tested. The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of lower body-weight gains at the next higher dose. Fludioxonil was not teratogenic in this study (Savary, 1989a).

Rabbits

Groups of 16 female rabbits were artificially inseminated and then treated with fludioxonil (purity, 97.5%) at a dose of 0, 10, 100 or 300 mg/kg bw per day by gavage in 0.5% methylcellulose solution in distilled water during days 6–18 of gestation. Maternal toxicity was evaluated using: rate of mortality, daily clinical observations, measurements of body weight and food consumption, and dissection and examination of the reproductive tract. Fetuses were removed by caesarean section on day 28 of gestation and toxicity was determined by external, visceral and skeletal examination, and measurement of body weight. Food, water and the housing environment were controlled and monitored. One female at 100 mg/kg bw per day died during the study and one dam in the control group, and in the groups receiving a dose of 10 and 300 mg/kg bw per day had total resorptions. One female in the control group and one female at 300 mg/kg bw per day aborted. Blue-coloured urine,

Table 20. Key observations in a study of developmental toxicity in rabbits given fludioxonil by gavage

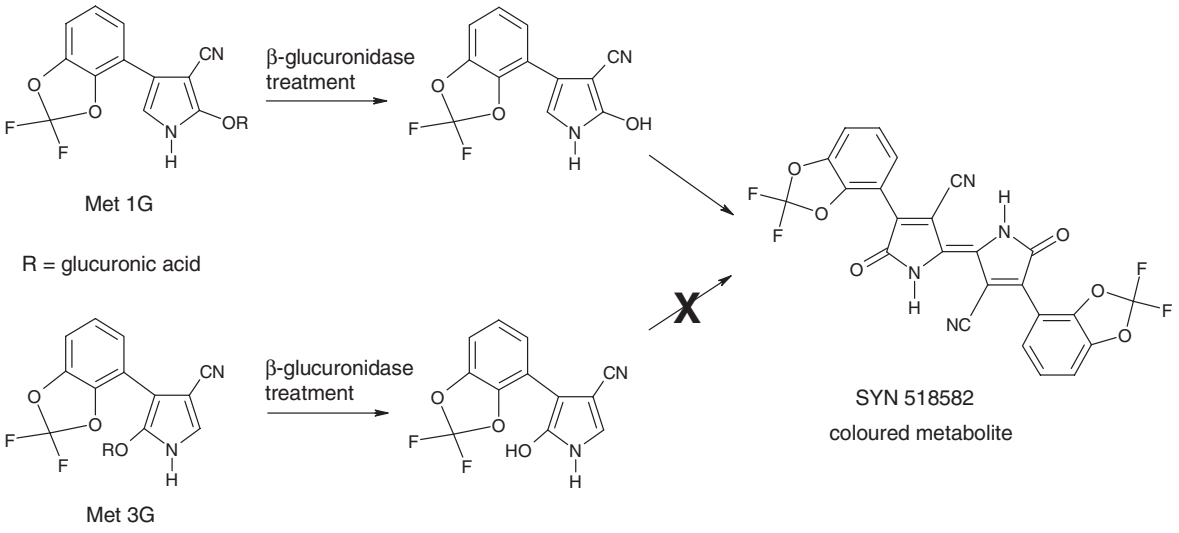
Observation	Dose (mg/kg bw per day)			
	0	10	100	300
Skeletal variation				
Reduced ossification of the fifth sternebra	22	20	30	33
Total skeletal variations	40	40	39	52
Body and organ weights				
Body weight (g/[% of control])				
Day 0	3763/100	3744/100	3716/99	3702/98
Day 6	3937/100	3946/100	3908/99	3861/98
Day 18	4089/100	4080/100	4034/99	3938/96
Day 28	4234/100	4205/99	4140/98	4061/96
Body-weight gain (g/[% of control])				
Days 0–6	173.3/100	140.7/81	191.4/110	159.3/92
Days 6–18	152.5/100	195.7/128	126.4/83	76.4/50
Days 18–28	145.0/100	125.0/86	105.7/73	122.9/85
Days 0–28 (individual range)	470.8/100	461.4/98	423.6/90	358.6/76
Days 0–28[a] (individual range)	63.6 (−514–+431)	47.3 (−302–+287)	9.6 (−393–+374)	−70.2 (−413–+201)
Uterus weight (g/[% of control])	407.2/100	414.1/102	414.0/102	428.8/105
Food consumption ([g/animal per day]/ [% ofcontrol]				
Days 0–6	209/100	193/92	198/95	189/90
Days 6–12	188/100	193/103	180/96	145*/77
Days 12–19	191/100	189/99	194/102	164/86
Days 19–24	181/100	165/91	171/95	153/85
Days 24–28	138/100	127/92	148/107	110/80

From Savary (1989b)
* $p < 0.05$ (Student's t-test)
[a] Without uterus weight

attributable to the presence of a blue metabolite of fludioxonil, was noted in four dams at 100 mg/kg bw per day and 12 dams at 300 mg/kg bw per day, but was considered to be of no toxicological relevance. Females treated with fludioxonil at 300 mg/kg bw had significantly lower body-weight gains during gestation (471, 424 and 359 g at 0, 100 and 300 mg/kg bw per day respectively), and reduced food consumption over the period of the study, and those at 100 mg/kg bw per day weighed sightly less than the controls from day 18. The equivocally lower net body-weight gain, without reduced food consumption, observed at 100 mg/kg bw per day was at least partially due to lower body weights in this group at the start of dosing. The individual values were well within the range of those for the controls and the final body weights expressed as a percentage of those of the controls were comparable at doses of up to 100 mg/kg bw per day (100%, 99%, 97% and 95% at 0, 100 and 300 mg/kg bw per day respectively). Consequently, the slightly lower body-weight gain at 100 mg/kg bw per day was considered to be not adverse and was probably incidental.

The sex ratio was statistically different in the control group (63% male) and at 300 mg/kg bw (48% male), but is unlikely to be attributable to treatment. Treatment did not affect the number of resorptions, fetal loss, fetal weights, or external, visceral and skeletal anomalies. There was a non-significant increase in the incidence of reduced ossification of the fifth sternebra and overall incidence of skeletal variations at 300 mg/kg bw, which is likely to reflect delayed development secondary to maternal nutritional stress manifested as reduced weight gains and food consumption. The NOAEL for fetal development was 300 mg/kg bw per day. The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of lower body-weight gains and reduced food consumption at the next higher dose (Savary, 1989b).

Figure 3. Mechanism of production of the coloured metabolite of fludioxonil



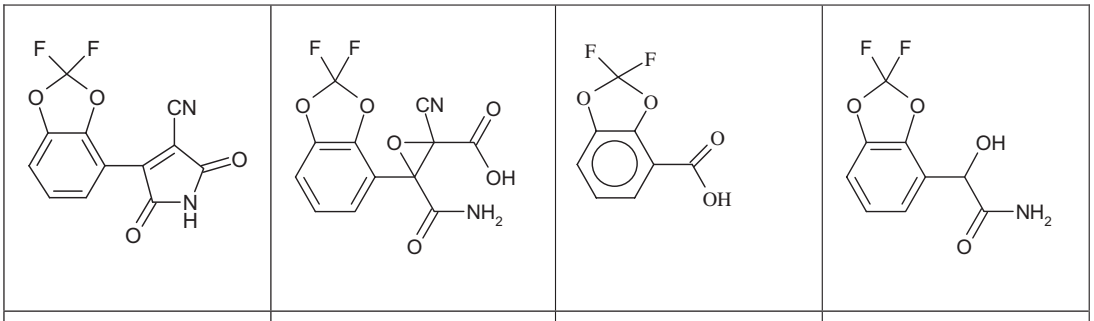
2.6 Special studies: mechanisms

To investigate the cause of the blue colouration seen in the urine and some organs, the metabolites of fludioxonil in pooled samples of urine collected at 1, 3, 6, 12, 18 and 24 months during the long-term study in rats given diets containing fludioxonil at a concentration of 0, 10, 30, 100, 1000 or 3000 ppm were investigated. Male rats given diets containing fludioxonil at 3000 ppm for 7, 13 and 24 months were given a pulse dose of [4-^{14}C]pyrrole fludioxonil by gavage. Urine was collected from these animals for the next 72 h. From the original study, animlas at 1000 and 3000 ppm displayed coloured urine with an absorption maximum of 594 ± 2 nm. The absorption spectrum was between 450 nm and 650 nm. Two of the original samples had a further peak at 650 nm. For this peak, absorption increased with storage, indicating that it is derived from a degradation product. The extent of colouration of the urine reached a plateau after 3 months. The proportion of the administered radiolabel that was recovered in the urine was similar at 7, 13 and 24 months (24–33% over 24 h and 30–34% over 72 h). In a previous study of metabolism, 16.5% of the administered radiolabel was collected in the urine over 72 h after a single dose of fludioxonil. The pattern of metabolites was determined for the 0–24 h after the pulse dose. For all samples, a pronounced absorption maximum was noted between 593 nm and 596 nm. The pattern of urinary metabolites was similar to that noted in toxicokinetic studies. One nonpolar fraction was revealed to be the coloured component and represented between 3.9 and 6.4% of the radiolabelled dose, which corresponds to 1–2% of a daily dose at a dietary concentration of 3000 ppm. When one of the other metabolites was treated with β-glucuronidase, the solution turned blue immediately. The relative molecular mass was determined to be 538 and the proposed structure is presented in Figure 3 (Thanei, 1994).

2.7 Studies on metabolites

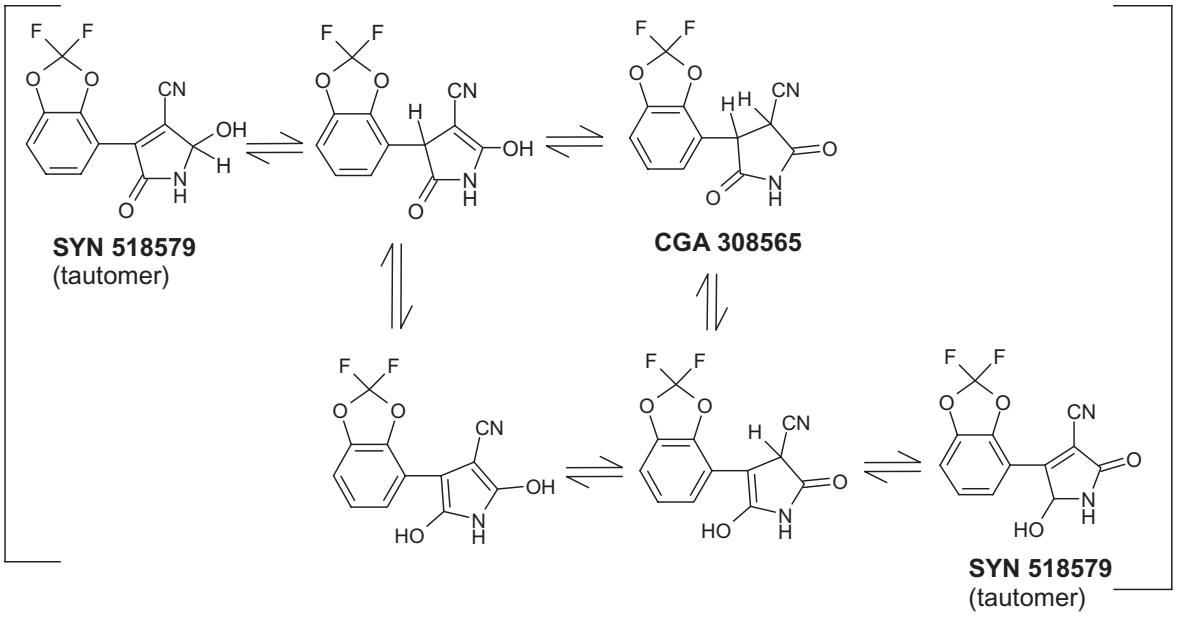
Studies were conducted on the acute oral toxicity and genotoxicity of several metabolites of fludioxonil that are formed in plants, soil, and/or chickens. These metabolites have the company codes of SYN 518579/CGA 308565 (a tautomeric pair; structures are given in Figure 4), CGA 265378, CGA 339833, CGA 308103, and CGA 192155. Their structures are provided in Figure 5, and the results of these studies are summarized in Table 21. In addition, a 90-day dietary study with CGA 339833 was conducted in rats.

Figure 4. Structures of some key plant and animal metabolites of fludioxonil that are not found in rats



CGA 265378	CGA 339833	CGA 192155	CGA 308103

Figure 5. Tautomerism of SYN 51879 and CGA 308565



CGA 339833 is formed from fludioxonil by photolysis, hydroxylation, and consequent opening of the pyrrol ring. The compound was identified in soil and water (photolysis studies at 15–31%) and in plants (wheat, peach, green onions, lettuce and in studies of rotational crops with wheat, radish and mustard).

Groups of 10 male and 10 female HanBrl:WIST(SPF) rats were given diets containing CGA 339833 (purity, 96%) at a concentration of 0, 10, 100, 800, 2500 or 7000 ppm for 90 days. Actual achieved doses of CGA 339833 were 0.7, 7.1, 58, 190, 510 mg/kg bw per day for males and 0.9, 8.7, 67, 210, 600 mg/kg bw per day. Stability and homogenicity of the test article in the diet were checked before treatment and at intervals during the study. Mortality was checked twice per day and clinical signs were checked daily. Body weight, food and water consumption were recorded before the start of treatment and once per week thereafter. Ophthalmological examinations were performed for all animals before treatment, and for animals in the control group and in the group receiving the highest dose in week 13. A functional observational battery (activity and overexcitation of the central nervous

Table 21. Acute oral toxicity and genotoxicity of metabolites of fludioxonil

Metabolite	End-point and test object	Dose	Result	Reference
Syn 518579/CGA 308565	LD_{50}, rat	2000 mg/kg bw	LD_{50} > 2000 mg/kg bw (no deaths)	Hartmann (1993a)
	Ames test, *S. typhimurium* and *E. coli*	312.5–5000 µg/plate ±S9	Negative	Hertner (1992c)
CGA 265378	LD_{50}, rat	2000 mg/kg bw	LD_{50} > 2000 mg/kg bw (no deaths)	Hartmann (1992)
	Ames test, *S. typhimurium* and *E. coli*	312.5–5000 µg/plate ±S9	Negative	Hertner (1992a)
CGA 339833	LD_{50}, rat	2000 mg/kg bw	LD_{50} > 2000 mg/kg bw (no deaths)	Sommer (2000)
	Ames test, *S. typhimurium* and *E. coli*	312.5–5000 µg/plate ±S9	Negative	Deparade (2000)
	Gene mutation, mouse lymphoma L5178Y cells	4 h: 400–3200 µg/ml (−S9); 200–3400 µg/ml (+S9); 24 h: 400–3200 µg/ml (−S9)	Negative	Wollny (2001)
	In vitro Chromosomal aberration, Chinese hamster V79 cells	−S9: 800–2400 µg/ml (4 h + 14 h), 200–1600 µg/ml (18 h), 800 µg/ml (28 h); +S9: 200–2400 µg/ml (4 h + 14 h), 200–800 µg/ml (4 h + 24 h)	Positive: −S9: 18 h Negative −S9: 4 h (+14 h) and 28 h +S9: 4 h (+14 h) and 4 h (+24 h)	Schulz (2002)
	In vivo Micronucleus formation, rat bone marrow	500, 1000 or 2000 mg/kg bw	Negative	Fox (2002)
CGA 308103	LD_{50}, rat	200, 500, 1000 or 2000 mg/kg bw	1000 < LD_{50} < 2000 mg/kg bw (Deaths: 0/5, 0/5, 0/3, 5/5)	Hartmann (1993c)
	Ames test, *S. typhimurium* and *E. coli*	312.5–5000 µg/plate	Negative	Hertner (1994)
CGA 192155	LD_{50}, rat	2000 mg/kg bw	LD_{50} > 2000 mg/kg bw	Hartmann (1993b)
	Ames test, *S. typhimurium* and *E. coli*	78.1–1250 µg/plate (+S9 *S. typhimurium* first experiment) 312.5–5000 µg/plate (all other experiments)	Negative	Hertner (1993b)

system, sensorimotor, autonomic and physiological functions and motor activity tests) was performed towards the end of treatment. At the end of treatment, haematology, clinical chemistry, urine analysis, organ weights, gross pathology and histopathology were performed.

There were no treatment-related effects on mortality, clinical signs, the functional observation battery, food and water consumption, haematology, ophthalmological parameters, or gross pathology. Body-weight gain was slightly reduced in animals at the highest dose, resulting in final body weights that were 10% and 5% lower than those of the controls for males and females respectively. Apparently lower body-weight gain in males at 100 ppm was at least partly due to single animals in the control group and in the group receiving fludioxonil at 10 ppm with cumulative weight gains that were >50% greater than the average for those groups. If body-weight gains are calculated without these two outliers, then values for groups of males treated at ≤100 ppm are similar to those of the controls. Weight gains for groups treated with fludioxonil at 800 to 7000 ppm remain approximately 10–13% below that of the control group, but with a flat dose–response

relationship particularly at 800 and 2500 ppm. Consequently, lower body-weight gains in males treated at 100 ppm are considered to be incidental to treatment and those observed at 800 and 2500 ppm to be of equivocal relationship to treatment. Significantly lower triglyceride and higher phosphorus values were observed in males at 7000 ppm. Males at 7000 ppm excreted slightly larger volumes of a more dilute urine. A slightly increased absolute weight of the liver was noticed in females at 7000 ppm and relative weights were increased in both sexes at this dose. The weight changes correlated with centrilobular hepatocellular hypertrophy. Relative weight of the kidney was increased for males at 7000 ppm and slight non-significant increases were seen in males at 2500 ppm and in females at 7000 ppm. Histopathologically, a slightly increased incidence and severity of tubular casts was seen in males at 800 to 7000 ppm. The tubular casts were found in the proximal tubuli and consisted of eosinophilic formations in the tubular lumen and walls without causing tubular distention. As tubular casts were present in more than half of the control males, the slight increase in incidence and grading was not considered to be adverse—especially for males at 800 ppm where this finding was not accompanied by any change in kidney weight or other indications of kidney damage in clinical pathology parameters. Increased relative weights of the testes in males at 7000 ppm were considered to be secondary to reduced weight gains in this group, as absolute testes weights tended to be preserved. Increased weights of the ovaries in females treated at 100 ppm were attributable to the presence of watery cysts in one animal, and increased adrenal weight in this group was discounted as incidental owing to the lack of a dose–response relationship. Histopathology revealed an increased incidence of minimal to slight atrophy in the olfactory epithelium at 2500 and 7000 ppm, which was characterized by the disorderly arrangement of the olfactory epithelium, retention of secretion in the olfactory mucosal glands, and foamy appearance of submucosal supporting cells. A slightly increased incidence of follicular cell hypertrophy in the thyroid gland was seen in females at 7000 ppm.

The NOAEL for CGA 339833 was 800 ppm (equal to 58 and 67 mg/kg bw per day in males and females) on the basis of increased relative weight of the liver correlating with hepatocellular hypertrophy, increased relative weight of the kidney and tubular casts at ≥2500 ppm (males only), and minimal to slight atrophy of the olfactory epithelium at ≥2500 ppm (Sommer, 2001).

3. Observations in humans

Medical surveillance of employees engaged in the manufacture of fludioxonil or its formulation into products since 1992 has not revealed any adverse health effects. Medical surveillance included, at some sites, anamnesis, physical examination, haematology clinical chemistry and urine analysis (Lorez & Ledgerwood, 2003).

Comments

After oral administration of radiolabelled fludioxonil, the radiolabel is rapidly and extensively (approximately 80% of the administered dose) absorbed, widely distributed, extensively metabolized and rapidly excreted, primarily in the faeces (approximately 80%) via the bile (approximately 70%), with a small amount being excreted in the urine (approximately 20%). The maximum blood concentration is reached within 1 h after administration. Elimination is biphasic, with half-lives of between 2 h and 5 h for the first phase, and between 30 and 60 h for the second phase. Fludioxonil is rapidly cleared from the blood and tissues, and there is consequently negligible potential for accumulation. The metabo-

Table 22. Principle findings of a 90-day study with CGA 339833 in rats

	Dietary concentration (ppm)											
	Males						Females					
	0	10	100	800	2500	7000	0	10	100	800	2500	7000
Terminal body weight (g)	403.5	398.5	374.0	371.6	374.1	359.8	215.6	222.4	216.4	220.6	212.1	205.4
Body weight (% of control), week 13	100	99	94	93	94	91	100	103	101	102	98	95
Body-weight gain (g), weeks 1–13	227	221	203	197	199	188**	93	98	97	93	86	81
Triglycerides (mmol/l)	0.84	0.79	0.71	0.62	0.54	0.45**	0.391	0.417	0.399	0.466	0.384	0.367
PO_4^- (mmol/l)	1.56	1.45	1.45	1.46	1.57	1.81**	1.27	1.22	1.54*	1.35	1.44	1.49
Urine												
Volume (ml)	2.98	3.66	2.63	3.67	4.02	4.69*	3.80	3.91	3.26	4.03	4.46	4.16
Relative density (1)	1.072	1.063	10.78	1.066	1.053	1.046*	1.050	1.048	1.051	1.036	1.040	1.046
Liver												
Absolute weight (g)	14.6	13.91	13.38	12.67	13.57	14.23	7.66	7.86	8.13	7.79	7.76	8.66
Relative to body weight (%)	36.2	35.1	35.8	34.1	36.4	39.5*	35.5	35.4	37.5	35.4	36.6	42.3*
Relative to body weight (% of control)	100	97	99	94	101	109	100	100	106	100	103	119
Hepatocyte hypertrophy [grade]	0/10 [—]	0/10 [—]	0/10 [—]	0/10 [—]	7/10 [1.0]	10/10 [2.4]	0/10 [—]	0/10 [—]	0/10 [—]	0/10 [—]	1/10 [1.0]	7/10 [1.9]
Kidney												
Absolute weight (g)	2.519	2.427	2.373	2.343	2.524	2.492	1.630	1.683	1.657	1.584	1.551	1.615
Relative to body weight (%)	6.25	6.14	6.35	6.30	6.78	6.93**	7.54	7.58	7.66	7.21	7.32	7.88
Relative to body weight (% of control)	100	98	102	101	109	111	100	101	102	96	97	105
Tubular casts [grade]	6/10 [1.3]	7/10 [1.3]	7/10 [1.0]	8/10 [1.8]	10/10 [1.6]	10/10 [1.8]	0/10 [—]	0/10 [—]	1/10 [2.0]	1/10 [2.0]	0/10 [—]	0/10 [—]
Nasal cavity												
Atrophy of olfactory epithelium [grade]	0/10 [—]	0/10 [—]	0/10 [—]	0/10 [—]	4/10 [1.0]	10/10 [1.6]	0/10 [—]	0/10 [—]	0/10 [—]	0/10 [—]	1/10 [1.0]	7/10 [1.9]
Thyroid gland												
Follicular cell hypertrophy [grade]	10/10 [1.2]	9/10 [1.3]	8/10 [1.3]	7/10 [1.0]	5/10 [1.0]	10/10 [1.4]	2/10 [1.0]	3/10 [1.0]	1/10 [1.0]	3/10 [1.0]	1/10 [1.0]	5/10 [1.0]

From Sommer (2001)
*$p < 0.05$, **$p < 0.01$ (Dunnett's test)

lism of fludioxonil proceeds primarily through oxidation of the pyrrole ring, leading to one major (57% of the administered dose) and one minor (4% of the administered dose) oxo-pyrrole metabolite. Hydroxylation of the phenyl ring yields the corresponding phenol metabolite, which represents 2% of the administered dose. These phase I metabolites are subsequently excreted as glucuronyl and sulfate conjugates and, together with unabsorbed and unchanged fludioxonil excreted in faeces, account for approximately 75% of the administered dose. The dimerization of the hydroxy pyrole metabolite produces a metabolite of an intense blue colour.

The dermal absorption of fludioxonil, excluding material bound to the skin, is low in rats in vivo (<5%) and in human skin in vitro (<0.5%). In a study of dermal penetration in rats in vitro, values for dermal absorption at low levels of application were comparable to those obtained in a study performed in vivo (<2%), but at higher levels these values significantly overestimated absorption in vivo (38%).

Fludioxonil has low acute toxicity in rats when administered by oral, dermal or inhalation routes, producing no deaths at 5000 and 2000 mg/kg bw and 2.6 mg/l of air, respectively, the highest doses tested. There were also no deaths in mice given fludioxonil at 5000 mg/kg bw by gavage. Fludioxonil is a slight ocular irritant in rabbits, but is neither a dermal irritant in rabbits nor a dermal sensitizer in guinea-pigs (Magnusson & Kligman maximization assay).

In studies of repeated doses in mice and rats, the liver (necrosis, centrilobular hypertrophy, increased serum cholesterol and 5'nucleotidase), the kidneys (nephropathy, inflammation, cysts) and haematopoietic system (mild anaemia) were the principle targets. Such effects often set the LOAELs for these studies, together with reduced body-weight gains. In mice, these effects were observed after 90 days of treatment at 450 mg/kg bw per day and at 590 mg/kg bw per day in one 18-month study, but not at 360 mg/kg bw per day in another such study. In rats, effects were seen at doses of ≥400 mg/kg bw per day in short-term studies and at 110 mg/kg bw per day in a 2-year study; lower body-weight gains were also observed at these doses. Liver toxicity was generally manifested by increased concentrations of serum cholesterol and bilirubin, and centrilobular hypertrophy and/or necrosis. Anaemia in mice (at >590 mg/kg bw per day for 18 months) and rats (at 1300 mg/kg bw per day for 3 months) was seen at doses greater than the LOAEL. In dogs, anaemia was observed at the LOAEL (at 290 mg/kg bw per day for 3 months, but only after 4 weeks of treatment; and at a dose of 300 mg/kg bw per day for 12 months). No haematological effects were observed in shorter studies in mice (at ≤1050 mg/kg bw per day for 90 days) or rats (at ≤2500 mg/kg bw per day for 20 days, and at ≤1000 mg/kg bw per day for 28 days).

Blue discolouration of the urine, perineal fur, kidneys and gastrointestinal tract were common observations in all species. These effects were secondary to the formation of the blue metabolite in quantities that were sufficient, at high doses, to stain the various tissues. The effect is not toxicologically significant and was disregarded in identifying NOAELs from studies in which it was observed.

Fludioxonil gave negative results in assays for reverse mutation in *S. typhimurium* and *E. coli*, gene mutation in Chinese hamster V79 cells, unscheduled DNA synthesis in rat hepatocytes, micronucleus formation in bone marrow of rats and mice in vivo, and chromosome aberration in Chinese hamsters in vivo. Fludioxonil was clastogenic in Chinese hamster ovary cells (CCL61) in vitro at non-cytotoxic concentrations. There was no evidence of heritable genetic damage in an assay for dominant lethal mutations in mice.

The Meeting concluded that fludioxonil is unlikely to be genotoxic in vivo.

The carcinogenicity potential of fludioxonil was examined in a study in rats and in two studies in mice. While the incidence of lymphomas was slightly increased in females in one study in mice receiving diets containing fludioxonil at a concentration of 3000 ppm (equal to 360 mg/kg bw per day), no increase was observed in a concurrent life-time study in mice given diets containing fludioxonil at dietary concentrations of up to 7000 ppm (1000 mg/kg bw per day). Lymphoma is a common finding in ageing female CD-1 mice, and the historical incidence at the laboratory conducting these studies was 13–32%, which encompasses the incidence noted in females at 3000 ppm (30%). Given the high background rate of this finding and the lack of any increase in the other study by the same authors using higher doses, the Meeting concluded that the apparent increase in lymphomas observed in one study in mice was incidental. There was no evidence of carcinogenic potential

with fludioxonil in the study in rats. The overall NOAELs in the long-term studies were 112 mg/kg bw per day and 37 mg/kg bw per day in mice and rats respectively.

On the basis of the above consideration and on the lack of genotoxic potential in vivo, the Meeting concluded that fludioxonil is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, at a dose of 210 mg/kg bw per day, adult males had reduced body-weight gains and food consumption and pups had lower body-weight gains than did the controls. The NOAEL for parental and pup toxicity was 21 mg/kg bw per day. The NOAEL for effects on reproductive performance was 21 mg/kg bw per day on the basis of reduced pup weights. As no effects on body-weight gain, or any other parameter, were seen in adult rats at 37 mg/kg bw per day in a 2-year study, the NOAEL for parental animals in the study of reproductive toxicity can also be considered to be >37 mg/kg bw per day. The NOAEL of 21 mg/kg bw per day for pup toxicity was based on effects observed at 210 mg/kg bw per day. These effects were relatively mild and the dose–response relationship appears to be shallow (12% decrease in body-weight gain over the dose range of 190 mg/kg bw per day, or a 1%, or less, decrease in body-weight gain per dose increment of 16 mg/kg bw per day). Assuming a linear dose–response relationship between 21 and 210 mg/kg bw per day, then at the proposed overall NOAEL for rats of 37 mg/kg bw per day, a decrease in body-weight gain of ≤1% would be predicted in pups; this would not be interpreted as being an adverse effect. Consequently, the use of an overall NOAEL of 37 mg/kg bw per day is also appropriate for pup toxicity. In a study of developmental toxicity in rabbits and another in rats, fludioxonil was neither teratogenic nor fetotoxic and fetal weights were unaffected at doses of up to 1000 mg/kg bw per day and 300 mg/kg bw per day, respectively. Maternal toxicity in these studies was limited to reduced body-weight gain at 1000 mg/kg bw per day and 300 mg/kg bw per day in rats and rabbits, respectively.

The Meeting concluded that the existing database on fludioxonil was adequate to characterize the potential hazards to fetuses, infants and children.

Studies of acute oral toxicity and genotoxicity with a range of plant metabolites of fludioxonil demonstrated that these metabolites were of low acute oral toxicity and were not genotoxic. A 90-day study in rats given diets containing a photolytic/hydrolytic degradation product of fludioxonil found in soil and water yielded a NOAEL of 800 ppm (equal to 58 mg/kg bw per day), on the basis of increased relative weight of the kidney and tubular casts at ≥2500 ppm (in males), and minimal to slight atrophy of the olfactory epithelium at ≥2500 ppm.

Toxicological evaluation

The Meeting established an ADI of 0–0.4 mg/kg bw based on a NOAEL of 37 mg/kg bw per day in a 2-year dietary study in rats, and a 100-fold safety factor.

Although effects on the kidneys occurred after relatively short periods of exposure, the Meeting concluded that such effects were unlikely to result from a single exposure. Consequently, the Meeting concluded that an ARfD for fludioxonil was unnecessary.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month study of toxicity and carcinogenicity[a]	Toxicity	1000 ppm, equal to 112 mg/kg bw per day	3000 ppm, equal to 360 mg/kg bw per day
		Carcinogenicity	3000 ppm, equal to 360 mg/kg bw per day[b]	—
Rat	2-year study of toxicity and carcinogenicity[a]	Toxicity	1000 ppm, equal to 37 mg/kg bw per day	3000 ppm, equal to 110 mg/kg bw per day
		Carcinogenicity	3000 ppm, equal to 110 mg/kg bw per day[b]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	300 ppm, equal to 21 mg/kg bw per day[d]	3000 ppm, equal to 210 mg/kg bw per day
		Embryo- and fetotoxicity	300 ppm, equal to 21 mg/kg bw per day[d]	3000 ppm, equal to 210 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	100 mg/kg bw per day	1000 mg/kg bw per day
		Embryo- and fetotoxicity	1000 mg/kg bw per day[b]	—
Rabbit	Developmental toxicity[c]	Maternal toxicity	100 mg/kg bw per day	300 mg/kg bw per day
		Embryo- and fetotoxicity	300 mg/kg bw per day[b]	—
Dog	12-month study of toxicity[a,e]	Toxicity	1000 ppm, equal to 33 mg/kg bw per day	8000 ppm, equal to 300 mg/kg bw per day

[a] Diet
[b] Highest dose tested
[c] Gavage
[d] The NOAEL of 21 mg/kg bw per day in this study was adjusted upwards to 37 mg/kg bw per day on the basis of an absence of effects in 90-day and 2-year studies in rats at 64 and 37 mg/kg bw per day, respectively. Additionally, interpolation of the reduced weight gain in pups in the study of reproductive toxicity indicated a likely, non-adverse, reduction in weight gain of 1% or less at the higher NOAEL of 37 mg/kg bw per day
[e] The LOAEL for this study was 300 mg/kg bw per day. Owing to the wide dose spacing used for this study, the NOAEL is conservative and hence the slightly higher NOAEL obtained in the 2-year study in rats was selected for establishment of the ADI

Estimate of acceptable daily intake for humans

0–0.4 mg/kg bw

Estimate of acute reference dose

Unnecessary

Studies that would provide information useful for continued evaluation of the compound

Further observations in humans

Summary of critical end-points for fludioxonil

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of oral absorption	Rapid, approximately 80%
Dermal absorption	Poor, <10% in the rat in vivo; ≤1% in human skin in vitro
Distribution	Extensive
Rate and extent of excretion	Largely complete within 24 h; approximately 10% in urine and 80% in the faeces; 70% of the administered dose was excreted in the bile
Potential for accumulation	Low, no evidence of accumulation
Metabolism in mammals	Extensively metabolized, involving primarily oxidation of the pyrrole ring leading to a major (57% of the administered dose) and a minor (4% of the administered dose) oxo-pyrrole metabolite, followed by glucuronyl- and sulfate conjugation
Toxicologically significant compounds (animals, plants and the environment)	Parent compound and metabolites
Acute toxicity	
Rat, LD_{50}, oral	>5000 mg/kg bw (no deaths)
Rat, LD_{50}, dermal	>2000 mg/kg bw (no deaths)
Rat, LC_{50}, inhalation	>2.6 mg/l of air (no deaths)
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Slight irritant
Skin sensitization	Not sensitizing (Magnusson & Kligman test)
Short-term studies of toxicity	
Target/critical effect	Damage to liver (rats and dogs) and kidney (mice and rats)
Lowest relevant oral NOAEL	1000 ppm, equal to 33 mg/kg bw per day (12-month study in dogs)
Lowest relevant dermal NOAEL	200 mg/kg bw per day (rats)
Lowest relevant inhalation NOAEC	—
Genotoxicity	Unlikely to pose a genotoxic risk in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body-weight gains and liver necrosis in rats, liver and kidney damage in mice
Lowest relevant NOAEL	1000 ppm, equal to 37 mg/kg bw per day, in rats
Carcinogenicity	Not carcinogenic in rats or mice; unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Reproductive target/critical effect	Reduced pup weight gains in rats at parentally toxic doses
Lowest relevant reproductive NOAEL	300 ppm, equal to 21 mg/kg bw per day
Developmental target/critical effect	None
Lowest relevant developmental NOAEL	300 mg/kg bw per day (the highest dose tested in rabbits)
Neurotoxicity	No evidence of neurotoxicity or delayed neurotoxicity in any study conducted
Other toxicological studies	Studies on plant metabolites and a photolytic/hydrolytic degradation product of fludioxonil indicated that these were of no greater toxicity than the parent compound
Medical data	Medical monitoring since 1992 of employees engaged in the manufacture of fludioxonil, or its formulation, into products has not revealed any adverse health effects

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.4 mg/kg bw	2-year study in rats (liver effects and reduced body-weight gains)	100
ARfD	Unnecessary	—	—

References

Bissig, R. (1990) Absorption, distribution, metabolism and excretion of [4-^{14}C] pyrrole CGA 173506 in the rat. Unpublished report No. 32/90 from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File No. CGA173506/0087.

Chang, J.C.F. (1990a) 90-Day oral toxicity study in rats. Unpublished report No. F-00014 from Environmental Health Center, Farmington, USA. Syngenta File No. CGA173506/0048.

Chang, J.C.F. (1990b) 90-Day oral toxicity study in mice. Environ. Health Center, Farmington, USA. Unpublished report No. F-00017 dated 31 January 1990. Syngenta File No. CGA173506/0050.

Chang, J.C.F. & Morrissey, R.L. (1990) 90-Day oral toxicity study in mice. Unpublished report No. F-00017 from Ciba-Geigy Corporation, Farmington, USA.

Chang, J.C.F. & Richter, A.G. (1993) 2-Year chronic toxicity/oncogenicity study in rats. Unpublished report No. F-00018 from Ciba-Geigy Corporation, Farmington, USA.

Chang, J.C.F. & Wyand, S.D. (1993a) 18-Month dietary oncogenicity study with CGA 173506 in mice. Unpublished report No. F-00019 from Environmental Health Center, Farmington, USA. Syngenta File No. CGA173506/0278.

Chang, J.C.F. & Wyand, S.D. (1993b) 18-Month dietary oncogenicity study with CGA 173506 in mice. Unpublished report No. F-00071 from Environmental Health Center, Farmington, USA. Syngenta File No. CGA173506/0279.

Courcy di Rosa, J. (1988) CGA 173506 technical—toxicity by oral administration to rats (admixture with the diet) for 20 days. Unpublished report No. 871518 (3548 TSR) from Centre International de Toxicologie, Evreux, France. GLPcompliant. Syngenta File No. CGA173506/0028.

Deparade, E. (1987) Salmonella cobas bact pilot test (study No. 861688). Unpublished report from Ciba-Geigy Ltd, Basel, Switzerland.

Deparade, E. (2000) CGA 339833 technical (metabolite of CGA 173506)—*Salmonella Escherichia*/mammalian-microsome mutagenicity test. Unpblished report No. 20003047 from Novartis Crop Protection AG, Basel, Switzerland. Syngenta File No. CGA339833/0007.

Dollenmeier, P. (1989) Point mutation test with Chinese hamster cells V79 (OECD conform). Unpublished report No. 881497from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File No. GA173506/0045.

Emeigh Hart, S.G. (1994) Amendment 2 to final report "2-Year chronic toxicity/oncogenicity study in rats (study report No. F-00018)" dated November 17 from Ciba-Geigy Ltd., Stein, Switzerland. Syngenta File No. CGA173506/5112.

Fankhauser, H. (1990) 28-Day oral cumulative toxicity study in rats (gavage). Unpublished report No. 881492 from Ciba-Geigy Ltd, Stein, Switzerland. Syngenta File No. CGA173506/0054.

Fox, V. (2002) CGA 339833 technical: rat bone marrow micronucleus test. Unpublished report No. SR1164 from Central Toxicology Laboratory, Cheshire, UK. Syngenta File No. CGA339833/0020.

Glaza, S.M. (1991a) Acute oral toxicity study of CGA 173506 technical in rats. Unpublished report No. HWI 10200144 from Hazleton Laboratories, Madison, USA. Syngenta File No. CGA173506/0103.

Glaza, S. (1991b) Primary dermal irritation study of CGA 173506 technical in rabbits. Unpublished report No. HWI 10200146 from Hazleton Laboratories, Madison, USA. Syngenta File No. CGA173506/0116.

Glaza, S. (1991c) Acute oral toxicity study of CGA 173506 technical in mice (project No. HWI 10200145). Unpublished report dated April 25 from Hazleton Wisconsin, Inc., Madison, Wisconsin, USA.

Glaza, S. (1991d) Primary eye irritation study of CGA 173506 technical in rabbits (project No. HWI 10200147A). Unpublished report dated June 14 from Hazleton Wisconsin, Inc, Madison, Wisconsin, USA.

Hartmann, H.R. (1988) Acute dermal toxicity in the rat with CGA 173506 technical. Unpublished report No. 881489 from Ciba-Geigy Ltd, Stein, Switzerland. Syngenta File No. CGA173506/0037.

Hartmann, H.R. (1989) Acute inhalation toxicity in the rat. Unpublished report No. 881491 from Ciba-Geigy Ltd, Stein, Switzerland. Syngenta File No. CGA173506/0038.

Hartmann, H.R. (1992) Acute oral toxicity in the rat. Unpublished report No. 923106 from Ciba-Geigy Ltd, Stein, Switzerland. Syngenta File No. CGA265378/0002.

Hartmann, H.R. (1993a) Acute oral toxicity in the rat. Unpublished report No. 923200 from Ciba-Geigy Ltd, Stein, Switzerland. Syngenta File No. CGA308565/0002.

Hartmann, H.R. (1993b) Acute oral toxicity in the rat. Unpublished report No. 933000 from Ciba-Geigy Ltd, Stein, Switzerland. Syngenta File No. CGA192155/0001.

Hartmann, H.R. (1993c) Acute oral toxicity in the rat. Unpublished report No. 933084 from Ciba-Geigy Ltd, Stein, Switzerland. Syngenta File No. CGA308103/0001.

Hassler, S. (1999) The *in vitro* percutaneous absorption of [phenyl-U-^{14}C] CGA 173506 formulated as SWITCH® 62.5 WG (A-9219 B) through rat and human epidermis. Unpublished report No. 023AM03 from Novartis Crop Protection AG, Basel, Switzerland. Syngenta File No. CGA173506/5098.

Hertner, T. (1989) Autoradiographic DNA repair test on rat hepatocytes (OECD conform). Unpublished report No. 881494 from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File No. CGA173506/0031.

Hertner, T. (1990) Micronucleus test, mouse. Unpublished report No. 881493 from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File NO. CGA173506/0047.

Hertner, T. (1992a) *Salmonella* and *Escherichia*/liver-microsome test. Unpublished report No. 923105 from Ciba-Geigy Basel, Genetische Toxikologie, Basel, Switzerland. Syngenta File No. CGA265378/0001.

Hertner, T. (1992b) Dominant lethal test, mouse, 8 weeks. Unpublished report No. 923068 from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File No. CGA173506/0228.

Hertner, T. (1992c) *Salmonella* and *Escherichia*/liver-microsome test. Unpublished report No. 923201 from Ciba-Geigy Basel, Genetische Toxikologie, Basel, Switzerland. Syngenta File No. CGA308565/0001.

Hertner, T. (1993a) Chromosome studies on somatic cells of Chinese hamster. Unpublished report No. 923099 from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File No. CGA173506/0241.

Hertner, T. (1993b) *Salmonella* and *Escherichia*/liver-microsome test. Unpublished report No. 933001 from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File No. CGA192155/0002.

Hertner, T. (1993c) In vivo/in vitro unscheduled and replicative DNA synthesis in rat hepatocytes. Unpublished report No. 933031 from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File No. CGA173506/0348.

Hertner, T. (1994) *Salmonella* and *Escherichia*/liver-microsome test. Unpublished report No. 933085 from Ciba-Geigy Basel, Genetische Toxikologie, Basel, Switzerland. Syngenta File No. CGA308103/0002.

Holbert, M.S. (1992) Acute inhalation study toxicity in rats (study No. 8391-91). Unpublished report dated January 6, Stillmeadow Inc., Sugar Land, Texas, USA.

Lorez, C. & Ledgerwood, K. (2003) Medical data. Unpublished report, dated March 25, from Syngenta Crop Protection AG, Basel, Switzerland. Syngenta File No. CGA173506/0645.

Mewes, K.E. (1999) Dermal absorption of [phenyl-U-^{14}C] CGA 173506 formulated as SWITCH® 62.5 WG (A-9219 B) in the rat. Unpublished report No. 023AM02 from Novartis Crop Protection AG, Basel, Switzerland. Syngenta File No. CGA173506/1396.

Meyer, A. (1991) In vivo micronucleus test on rat hepatocytes. Unpublished report No. 901145 from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File No. CGA173506/0089.

Moysan, F. (1990) Toxicity study by repeated oral administration for 13 weeks followed by a 28-day recovery period in Beagle dogs. Unpublished report No. 881173 (4279 TCC) from Centre International de Toxicologie, Evreux, France. Syngenta File No. CGA173506/0049.

Müller, T. & Thanei, P. (1995) Absorption and distribution kinetics of (4-^{14}C)pyrrole CGA 173506 in the rat after oral administration. Unpublished report No. 4/95 from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File No. CGA173506/0555.

Myhr, B.C. (1999) CGA 173506 technical—Evaluation of aneuploidy in rat bone marrow cells. Unpublished report No. 19965-0-458/1185-98 from Covance Laboratories, Vienna, USA. Syngenta File No. CGA173506/5139.

Ogorek, B. (1987) Salmonella mutagenicity test with three strains (study No. 861585). Unpublished report dated January 26, from Ciba-Geigy Ltd, Basel, Switzerland.

Ogorek, B. (1989) Salmonella/mammalian-microsome mutagenicity test (OECD conform). Unpublished report No. 881495 from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File No. CGA173506/0044.

Ogorek, B. (1999) CGA 173506 technical—In vivo micronucleus test on rat hepatocytes. Unpublished report No. 983167 from Novartis Crop Protection AG, Basel, Switzerland. Syngenta File No. CGA173506/5055.

Savary, M.H. (1989a) Assessment of possible embryotoxic or teratogenic effects in rats by oral route. Unpublished report No. 4517 RSR/CG 881177 from Centre International de Toxicologie, Evreux, France. Syngenta File No. CGA173506/0051.

Savary, M.H. (1989b) Assessment of possible embryotoxic or teratogenic effects in rabbits by oral route. Unpublished report No. 4801 RSL/CG 881728 from Centre International de Toxicologie, Evreux, France. Syngenta File No. CGA173506/0052.

Schneider, M. (1988a) Acute dermal irritation/corrosion study in the rabbit. Unpublished report No. 881487 from Ciba-Geigy Ltd, Stein, Switzerland. Syngenta File No. CGA173506/0040.

Schneider, M. (1988b) Acute eye irritation/corrosion study in the rabbit. Unpublished report No. 881486 from Ciba-Geigy Ltd, Stein, Switzerland. Syngenta File No. CGA173506/0039.

Schneider, M. (1988c) Skin sensitization test in the guinea pig (maximisation test). Unpublished report No. 881490 from Ciba-Geigy Ltd, Stein, Switzerland. Syngenta File No. CGA173506/0041.

Schneider, M. (1990) 28-Day repeated dose dermal toxicity study in the rat. Unpublished report No. 881488 from Ciba-Geigy Ltd, Stein, Switzerland. Syngenta File No. CGA173506/0083.

Schulz, M. (2002) In vitro chromosome aberration test in Chinese hamster V79 cells with CGA 339833 technical (metabolite of CGA 173506). Unpublished report No. 680503/20003058 from RCC Cytotest Cell Research GmbH, Rossdorf, Germany. Syngenta File No. CGA339833/0019.

Singh, A.R. (1992) A two-generation reproductive toxicity study in rats. Unpublished report No. MIN 902001 from Ciba-Geigy Corp., Summit, USA. Syngenta File No. CGA173506/0180.

Sommer, E.W. (2000) CGA 339833 technical (metabolite of CGA 173506)—Acute oral toxicity in the rat (limit test). Unpublished report No. 20003046 from Novartis Crop Protection AG, Stein, Switzerland. Syngenta File No. CGA339833/0003.

Sommer, E.W. (2001) CGA 339833 technical (metabolite of CGA 173506)—90-Day oral toxicity study in rats (administration in food). Unpublished report No. 20003048 from Syngenta Crop Protection AG, Stein, Switzerland. Syngenta File No. CGA339833/0015.

Strasser, F.F. (1989) Chromosome studies on Chinese hamster ovary cell line CCL 61 *in vitro* (OECD conform). Unpublished report No. 881496 from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File No. CGA173506/0046.

Thanei, P. (1992) Metabolic pathways of [4-^{14}C] pyrrole CGA 173506 in the rat. Unpublished report No. 12/92 from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File No. CGA173506/0199.

Thanei, P. (1994) On the nature and the extent of the urine coloration observed after subchronic and chronic administration of CGA 173506 to rats. Unpublished report No. 13/93 from Ciba-Geigy Ltd, Basel, Switzerland. Syngenta File No. CGA173506/0412.

Vallet, L. (1992) Toxicity study by repeated oral (dietary) administration for 52 weeks in Beagle dogs. Unpublished report No. 5577 TCC/CG 881174 from Centre International de Toxicologie, Evreux, France. Syngenta File No. CGA173506/0173.

Wollny, H.E. (2001) Cell mutation assay at the thymidine kinase locus (TK+/−) in mouse lymphoma L5178Y cells with CGA 339833 technical (metabolite of CGA 173506). Unpublished report No. 680501/20003059 from RCC Cytotest Cell Research GmbH, Rossdorf, Germany. Syngenta File No. CGA339833/0016.

FOLPET (addendum)

First draft prepared by
G. Wolterink and M.T.M. van Raaij
Centre For Substances and Integrated Risk Assessment,
National Institute of Public Health and the Environment,
Bilthoven, Netherlands

Explanation

Folpet is a fungicide used for the control of fungal diseases in crops. The Meeting prepared toxicological monographs on folpet in 1969 and 1995, and addenda to the monographs were prepared in 1973, 1984, 1986 and 1990. In 1995, an acceptable daily intake (ADI) of 0–0.1 mg/kg bw was established on the basis of a no-observed-adverse-effect level (NOAEL) of 10 mg/kg bw per day in a 2-year study of toxicity and carcinogenicity in rats, a 1-year study of toxicity in dogs, and studies of reproductive toxicity in rats and rabbits, and using a safety factor of 100. The present Meeting considered the requirement for an acute reference dose (ARfD) for folpet, based on data from the previous evaluations made by the Meeting and from new studies.

Evaluation for acute reference dose

1. Biochemical aspects: biotransformation

In vitro

In a study from the published literature, the degradation of folpet (purity, 94.6%) after incubation with human blood was investigated. Folpet at a concentration of about 1 µg/ml was mixed with blood at 37 °C. At various time-points ranging from 0 to 31 s, the reaction was terminated by adding phosphoric acid and acetone. Degradation of folpet and formation of phthalimide were measured.

Folpet was metabolized rapidly to phthalimide. The calculated half-life was 4.9 s. Mass spectrometry revealed that the phthalimide was the only degradation product (Gordon et al., 2001).

2. Toxicological studies

2.1 Acute toxicity

Mice

Two studies with folpet (purity, 96.9%) were performed to establish a NOAEL for gastrointestinal tract irritation in CD1 mice. Statements of adherence to quality assurance (QA) and good laboratory practice (GLP) were provided.

In a pilot experiment, groups of three female mice were given diets containing folpet at a concentration of 0, 200 or 5000 ppm (equal to 31 and 845 mg/kg bw) for 24 h. A fourth group of three females, fed on control diet, also received folpet at a dose of 1430 mg/kg bw by gavage. Body weight, food consumption and clinical signs were measured. After 24 h, the animals were killed and, after exsanguination, were injected via the tail vein with Evans Blue dye in order to visualize mucosal damage. The gastrointestinal tract was examined macroscopically. Stomach and duodenum were examined micropscopically.

Food consumption was reduced by 20–30% in all groups treated with folpet. In the animals treated by gavage, slight erosion and epithelial degeneration of stomach and slight mucosal congestion was observed. In the proximal duodenum, focal areas of moderate epithelial erosion and degeneration were observed. Occasionally, moderate loss of villi and congestion of mucosal vascularity was seen. In the proximal duodenum of animals at 5000 ppm, minimal to moderate epithelial erosions and degeneration were observed, but no abnormalities were detected in the stomach. One animal had moderate loss of villi and another mouse had slight mucosal congestion. No marked macroscopic and microscopic effects were observed in animals at 200 ppm (Moore, 200).

In the second study, groups of 15 female CD1 mice (or five in the control groups) received food containing folpet at a concentration of 0, 50, 200, 500 or 5000 ppm (equal to 0, 10, 44, 123 or 1060 mg/kg bw) for 24 h. An additional group fed on control diet that did not contain folpet was given folpet at a dose of 815 mg/kg bw by gavage. After 24 h, all animals were given control diet for the rest of the study. Animals were checked for clinical signs. Body weight was recorded at days 1, 3 and 7. Food consumption was measured during the first 24 h of the study. Five animals per group were killed on days 1, 3 and 7. From the control group, 3, 1 and 1 animals were killed on days 1, 3 and 7 respectively. In this study the animals did not receive Evans Blue dye. The gastrointestinal tract of all animals was examined macroscopically. Tissue from the stomach and duodenum obtained from animals killed at 24 h was examined microscopically.

Food consumption on day 1 was markedly reduced (44% of that of control animals) in animals given folpet at a dose of 815 mg/kg bw by gavage. Slight reductions (±10%) were observed in animals in the groups receiving diets containing folpet at 50, 200 and 5000 mg/kg of diet, while food consumption was not reduced at 500 mg/kg. At 815 mg/kg bw, one animal displayed decreased activity and was cold to touch on day 1. No other clinical signs were observed. Necropsy revealed no gross abnormalities in any of the groups at days 1, 3 and 7. Microscopical examination of the stomach and duodenum of the animals killed after one day revealed that two animals in the group at 815 mg/kg bw each had a single minimal erosion in the fundic stomach. No microscopic effects were observed in the stomach and proximal duodenum in mice fed for 24 h with diets containing folpet at a concentration of 5000 ppm (Moore, 2004).

The Meeting noted that in the second study, histopathology was only performed on day 1. It is not clear whether at this time, i.e. immediately after the end of the 24 h treament period, any histopathological changes would have fully developed.

2.2 Reproductive toxicity: developmental toxicity

Hamsters

In a study from the published literature, the teratogenic effects of a number of derivatives of phthalimide, including folpet, were tested in groups of two to eight pregnant golden hamsters given folpet as a single dose at 400, 500, 600, 700, 800, 900 or 1000 mg/kg bw on day 7 or 8 of gestation, or daily at a dose of 200, 300, 400 or 500 mg/kg bw per day from days 6 to 10 of gestation. Groups of dams serving as controls received either no treatment (n = 43) or carboxymethyl cellulose at a dose of 10 ml/kg bw (n = 99). The animals were killed and examined on day 15 of gestation. In the study report, the doses for the groups receiving folpet as repeated doses for 6–10 days are expressed as total dose. It is assumed that this total dose was administered evenly over the 5 days of treatment, with one treatment per day.

In the groups treated with folpet, maternal mortality occurred after all single doses except 500 mg/kg bw, and after repeated doses of ≥300 mg/kg bw per day. No terata were reported in the groups receiving repeated doses. In the groups treated with a single dose of folpet at 600 or 900 mg/kg bw on day 7 of gestation, an increase in the incidence of exencephaly was observed. There was no dose–response relationship. Exencephaly was not observed in animals treated with a single dose of folpet on day 8 of gestation, although one fetus (out of 43 fetuses examined) at 500 mg/kg bw had a cleft palate (versus none among the controls). Occasionally other skeletal malformations (short or curved tail, fused ribs, cranial pimple, limb defects) were observed in animals treated with single doses of folpet on day 7 or day 8 of gestation. No dose–response relationship was found. The study did not comply with GLP (Robens, 1970).

The Meeting noted that the mortality in the groups treated with repeated doses of folpet appeared to be related to dose. In the groups treated with a single dose, the relationship between mortality and dose was less clear. For the observed and skeletal effects of folpet in the golden hamster, no clear dose–response relationships were observed. This may be related to the small numbers of litters and fetuses in the treatment groups. Nevertheless, the incidences in exencephaly on day 7 were clearly higher than control values. Apart from the data on mortality, and the statement that diarrhoea occurred in many of the treated dams, no information about maternal toxicity was detailed in the paper. The Meeting considered that the mortality and developmental toxicity observed in this study was toxicologically relevant. Owing to the variability of the data, NOAELs for the maternal and fetal effects of folpet could not be established. The Meeting noted that this study has major limitations (e.g. small number of animals per dose, limited reporting of the data) and is therefore of limited value. It does, however, suggest that developmental effects may occur after a single exposure to folpet, albeit at maternally toxic doses.

Rats

In a pilot study, groups of eight female mated Crl:COBS CD(SD)BR rats were given folpet at a dose of 0, 20, 80, 320, or 640 mg/kg bw by gavage on days 6 to 19 of gestation. There were no deaths, but clinical signs including rales, excess salivation,

chromorhinorrhoea, gasping, soft or liquid faeces, decreased motor activity, dyspnoea, and distended gut were observed. Reduced maternal body weight was seen at ≥80 mg/kg bw, and food consumption was reduced at ≥320 mg/kg bw; the average fetal body weight was also reduced at the latter dose (Christian & Hoberman, 1983a; Annex 1, reference 43).

Groups of 25 CRL:COBS CD(SD)BR rats were given folpet (purity, 89%) at a dose of 0, 10, 60, or 360 mg/kg bw by gavage on days 6 to 19 of gestation. The vehicle was deionized water containing Tween 80 (0.5%) and carboxy methyl cellulose (0.7%). Rats were observed for clinical signs of toxicity, abortion, death, body-weight change and food consumption. Animals were killed on day 20. A statement of compliance with QA was provided.

Clinical signs consisting of rales (at 60 and 360 mg/kg bw per day) and excess salivation, chromorhinorrhoea, decreased motor activity, soft or liquid faeces, dyspnoea, and urine-stained fur (at 360 mg/kg bw per day) were observed. None of these effects were observed on the first day of treatment. Three rats at the highest dose died, two from intubation errors. No gross lesions attributable to treatment were seen in the surviving rats. In dams at the highest dose, significant reductions in body-weight gain (30%) and food consumption (15%) were observed from days 6 to 19 of gestation. The numbers of implantations, live and dead fetuses, fetal viability, and resorptions, the average fetal body weight per litter, the fetal sex ratio, and the number of corpora lutea were similar in all groups. There were no differences in gross external, visceral, and skeletal abnormalities and on skeletal ossification. The NOAEL for maternal toxicity was 60 mg/kg bw per day on the basis of reduced body-weight gain and food consumption and the increased incidence of clinical signs at 360 mg/kg bw per day. The NOAEL for embryo/fetotoxicity was 360 mg/kg bw per day, the highest dose tested (Christian & Hoberman, 1983b).

Groups of 22 female Charles River CD rats received folpet (purity, 91.1%) at a dose of 0, 150, 550, or 2000 mg/kg bw in 0.5% acetic acid containing 0.5% carboxymethylcellulose by gavage on days 6 to 15 of gestation. The animals were sacrificed on day 20. A QA statement was provided.

One animal in the group receiving folpet at the highest dose died on day 16 of gestation because of multiple haemorrhagic ulcerations of the gastric mucosa. Clear signs of toxicity were observed in animals at the highest dose, including soft faeces (in 21 out of 21 rats), fur staining (in 4 out of 21 rats), and perianal staining (in 8 out of 21 rats). Food consumption was decreased by 16% during the first days of treatment at the intermediate dose and was markedly decreased (27%) throughout treatment at the highest dose. Maternal body-weight gain from days 6 to 20 of gestation was also decreased by 19 and 28% at the intermediate and highest dose, respectively. Gravid uterine weights were depressed in dams at the middle and highest doses, but terminal maternal body weight (without the gravid uterus) was significantly depressed only at the highest dose. On the basis of the effects on body weight and food consumption, the NOAEL for maternal toxicity was 150 mg/kg bw per day. Pre- and postimplantation losses in animals at the intermediate dose were greater than those of the controls. Fetal weights were reduced at the intermediate and highest doses. Fetal crown–rump length was slightly decreased after treatment at the intermediate and highest doses. A single fetus (1 out of 277) at the highest dose had multiple major malformations, and a second pup had unilateral microphthalmia. The incidence of hepatic discolouration was significant at the highest dose. The incidences of angulated ribs, reduced ossification of interparietal bone, cranial and pubic bones, sternebrae, metacarpals, and

metatarsals were significantly increased in animals at the intermediate and highest doses. There was a slight increase in the incidence of angulated ribs and reduction of ossification of the parietal bone at 150 mg/kg bw per day. (Rubin & Nyska, 1985a).

In a study of developmental toxicity, that complied with guideline OECD 414, groups of 22 pregnant female CD rats were given folpet (purity, 93.7 %) at a dose of 20, 100 or 800 mg/kg bw per day by gavage from days 9 to 19 of gestation. Control animals received vehicle only (0.5% Tween 80 and 0.7% w/w carboxymethylcellulose). Females were killed on day 20 of gestation and uterine contents and fetuses were examined for abnormalities. Statements of compliance with QA and GLP were provided.

No deaths occurred. Apart from increased salivation during week 2 in dams at 800 mg/kg bw per day, no clinical signs were observed. Body-weight gain was reduced significantly at the highest dose. Food consumption in this group was reduced during days 6 to 8 and 15 to 17. No treatment-related effects were observed in dams at the lowest and intermediate doses. There was no effect of treatment on fetal growth and development. On the basis of reduced body-weight gain at highest dose, the NOAEL for maternal toxicity was 100 mg/kg bw per day. The NOAEL for fetal toxicity was 800 mg/kg bw per day, the highest dose tested (Myers, 2003).

Rabbits

Groups of 20 artificially inseminated New Zealand White rabbits were given folpet (purity, 89%) at a dose of 0, 10, 20, or 60 mg/kg bw by oral intubation on days 6 to 28 of gestation and were killed on day 29. The test article was suspended in vehicle containing Tween 80 and carboxymethylcellulose (concentrations not specified). A QA statement was provided.

The death of one doe at 60 mg/kg bw was considered to be related to treatment. One doe at the lowest dose aborted on day 21 of gestation and one at the highest dose on day 22 of gestation; one doe in the control group delivered a litter on day 28 and one at the highest dose on day 29. From days 6 to 29 of gestation, significant inhibition of body-weight gain was seen in animals at the intermediate (−0.2 kg) and highest doses (−0.4 kg) compared with controls (+0.19 kg). Effects were greatest from days 6 to 9 and days 12 to 18. Food consumption was correspondingly reduced. The average numbers of corpora lutea, implantations, resorptions, and fetuses per litter and the sex ratio and numbers of dead and resorbed implantations per litter were similar in all groups. The mean body weights of fetuses were decreased at the intermediate (11%) and highest dose (5%). A significant increase in the incidence of hydrocephaly (4 out of 65 fetuses in 3 out of 11 litters) was found at the highest dose. One fetus with hydrocephaly came from the litter of the doe that was found dead on day 27. These four fetuses also had skull, gastric, and pulmonary abnormalities. The observation of one fetus at the intermediate dose with hydrocephaly and cleft palate was considered to be within the range for historical controls (incidence of hydrocephalus in historical control was 3 out of 285 litters, 3 out of 2160 fetuses, and incidence of cleft palate was 2 out of 285 litters, 2 out of 2160 fetuses). The NOAEL for maternal and embryo/fetotoxicity was 10 mg/kg bw per day (Feussner et al., 1984).

In a preliminary study, groups of six mated HY/CR female New Zealand White rabbits were given folpet (purity, 91.1%) at a dose of 0, 10, 60, or 150 mg/kg bw per day by gavage on days 6 to 18 of gestation. Marked body-weight loss was seen at the highest dose.

Body-weight gain was reduced in the groups receiving folpet at a dose of 60 or 10 mg/kg bw per day. Although fetal size was unaffected by treatment, fetal mortality was more marked at the highest dose. Postimplantation losses were increased in animals at the intermediate dose (Rubin & Nyska, 1985b; Annex 1, reference 49)

Subsequent to this study, groups of 14 mated HY/CR New Zealand White rabbits were given folpet (purity, 91.1%) at dose of 0, 10, 40, or 160 mg/kg bw per day by gavage on days 7 to 19 of gestation. The vehicle contained 0.5% carboxymethylcellulose and 0.5% acetic acid. Dams were sacrificed on day 29 of gestation. A statement of compliance with QA was provided.

After 2 days of treatment, body-weight was decreased by 48 g and 159 g at the intermediate and highest dose respectively (in control animals, body-weight gain was 13 g). From day 10 onwards, body weights in animals at the intermediate dose had recovered to control values. Body weights of animals at the highest dose remained lower than those of controls for the entire period of gestation. Food consumption was decreased during days 7 to 10 and days 7 to 19 at the intermediate and highest dose respectively. There were no deaths. Gravid uterine weight was significantly reduced in dams at the intermediate (17%) and highest dose (19%). Fetal death (postimplantation loss) occurred more frequently at the highest dose (22%) than in controls (14%); the proportion of small fetuses was also increased in this group, and mean fetal weight was non-significantly reduced by 7%. There was evidence of a dose-related delay in skeletal maturation at the intermediate and highest doses (% of fetuses with reduced ossification incidences in control, intermediate and highest dose groups were: in caudal vertebrae, 0, 2 and 6%; in sternebrae 1–4, 0.8, 7 and 10%; in long-bone epiphyses, 21, 25 and 43%). The incidence of bilateral lumbar ribs was increased in animals at the highest dose. Other minor skeletal malformations did not appear to be related to treatment. There was no evidence of hydrocephalus in either treated or control rabbits. The NOAEL for maternal toxicity and embryo/fetotoxicity was 10 mg/kg bw per day (Rubin, 1985).

Groups of 20 artificially inseminated female Hazelton Dutchland New Zealand White (D1A Hra:(NZW) specific pathogen-free) rabbits were given folpet (purity, 89.5%) in a volume of 5 ml/kg bw per day by gavage in 0.5% Tween 80 (w/w) and 0.7% carboxymethylcellulose (w/w), to give a dose of 60 mg/kg bw per day on days 7 to 9, 10 to 12, 13 to 15, or 16 to 18 of gestation. Analysis of the formulations indicated that the folpet content was 87.8–104% of the nominal concentration. Dams were sacrificed on day 29 of gestation; those that aborted or delivered, the single animal that died, and those terminally sacrificed were subjected to necropsy and examination of the uterine contents. Statements of compliance with GLP and QA were provided.

One doe in the group receiving folpet on days 7 to 9 aborted one fetus and died on day 25 of gestation. Necropsy revealed three dead fetuses and three early resorptions in utero. One doe in the group receiving folpet on days 10 to 12 aborted on day 18 of gestation. These abortions may have been related to treatment with folpet. The incidence of soft or liquid faeces was increased in all treated groups, usually after treatment. Maternal body weight was significantly reduced during the respective treatment periods in all treated animals. Body-weight reductions over the 3 days of treatment were 0.08, 0.04, 0.11 and 0.17 kg in the groups receiving folpet on days 7 to 9, 10 to 12, 13 to 15 and 16 to 18 respectively. Food consumption was correspondingly reduced. After cessation of treatment with folpet, a quick recovery of maternal body weight and food consumption was observed in

the does in groups receiving folpet on days 7–9 and 10–12. In does in groups receiving folpet on days 13 to 15 and 16 to 18, recovery of body weight and food consumption was slower. No gross lesions attributable to treatment were seen at necropsy. Treatment had no apparent effect on the rate of abortion or on fetal resorption. The average litter sizes were unaffected, as were the average fetal weights, the number of viable fetuses, and the sex ratio. Two fetuses with hydrocephalus were observed, one in the group receiving folpet on days 10 to 12, and one in in the group receiving folpet on days 16 to 18. The incidence of hydrocephalus in historical controls were 5 out of 250 litters and 5 out of 1879 fetuses. A significantly increased incidence (12.1%) of fetuses with an irregularly shaped fontanelle was observed in the group treated on days 13 to 15; the control incidence was 4.5%. This variation did not occur in groups treated on days 7 to 9 or 16 to 18. It was possibly related to treatment, but the significance of the effect was not clear. Incidences for this effect among the historical controls were 5 out of 250 litters and 6 out of 1879 fetuses. There were no other significant variations in fetal skull morphology, and the incidence of gastric or pulmonary anomalies were not increased in any group (Feussner et al., 1985).

Comments

In the evaluation of folpet by JMPR 1995 (Annex 1, reference 74), it is reported that in rodents treated orally, folpet is rapidly degraded to phthalimide and thiophosgene (via thiocarbonyl chloride). Studies of metabolism in vitro with human blood revealed that folpet is rapidly degraded to phthalimide, with a calculated half-life of 4.9 s. Thiophosgene is rapidly detoxified by reaction with cysteine or glutathione, for example, and is ultimately rapidly excreted.

The acute toxicity of folpet in rats treated orally is low (LD_{50}, > 2000 mg/kg bw). In a study in pregnant hamsters, mortality occurred after a single dose at 400 mg/kg bw. In groups of pregnant New Zealand White rabbits treated with folpet at a dose of 60 mg/kg bw per day for 3 days, mortality was observed that may have been related to treatment.

Mice fed diets containing folpet at a concentration of 5000 ppm, equal to 845 or 1060 mg/kg bw, for 24 h showed a reduction in food consumption of 10–20%. Immediately after the 24 h of treatment, minimal to moderate epithelial erosions and degeneration of the proximal duodenum were observed in some of these animals. Microscopy revealed moderate loss of villi and slight mucosal congestion in some animals. Microscopy was not performed at later time-points.

Studies of developmental toxicity with folpet have been carried out in hamsters, rats and rabbits.

In a study from the published literature, the teratogenic effects of a number of phthalimide derivatives, including folpet, were tested in pregnant golden hamsters. The Meeting noted that this study has major limitations (e.g. small number of animals per dose, limited reporting of the data) and is therefore of limited value. It does, however, suggest that developmental effects may occur after a single exposure to folpet, albeit at maternally toxic doses.

Folpet has been tested in a number of studies of developmental toxicity in rats. In a study in Sprague-Dawley rats treated by gavage, the NOAEL for maternal toxicity was 60 mg/kg bw per day on the basis of reduced body-weight gain and food consumption and

increased incidence of clinical signs at 360 mg/kg bw per day. The NOAEL for embryo/fetotoxicity was 360 mg/kg bw per day, the highest dose tested. In another study in Sprague-Dawley rats treated by gavage, pregnant females received folpet at a dose of 0, 150, 550, or 2000 mg/kg bw on days 6 to 15 of gestation. On the basis of effects on body weight and food consumption, the NOAEL for maternal toxicity was 150 mg/kg bw per day. There was a slightly increased incidence of angulated ribs and the reduction in ossification of the interparietal bone at 150 mg/kg bw per day. The maternal toxicity and the associated fetal effects are likely to be caused by high local concentrations of folpet and are not considered to be relevant to dietary exposure. In a third study in Sprague-Dawley rats treated by gavage, the NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of reduced body-weight gain in the group receiving the highest dose. As treatment had no effect on fetal growth and development, the NOAEL for developmental toxicity was 800 mg/kg bw per day, the highest dose tested. Therefore, in these three studies of developmental toxicity in rats, the overall NOAEL for maternal toxicity was 150 mg/kg bw per day on the basis of reduction of body-weight gain and food consumption. In two out of three studies, no fetal developmental anomalies were found at doses of up to 800 mg/kg bw per day. In one study, however, a possible slight increase in developmental anomalies was reported at 150 mg/kg bw per day.

Folpet has been tested in a number of studies of developmental toxicity in rabbits treated by gavage. In a study in which New Zealand White rabbits were given folpet at a dose of 0, 10, 20, or 60 mg/kg bw per day on days 6 to 28 of gestation, the NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of reduced body-weight gain and food consumption. The NOAEL for fetal toxicity was 10 mg/kg bw per day on the basis of reduced fetal body weights. The maternal toxicity and the associated reduction in fetal body weight are likely to be caused by high local concentrations of folpet and are not considered to be relevant to dietary exposure. At 60 mg/kg bw per day, there was a significant increase in the incidence of hydrocephaly in four fetuses out of three litters. In these same fetuses, skull, gastric, and pulmonary abnormalities were also observed. As the observation of hydrocephaly and cleft palate in one fetus at the intermediate dose was considered to be within the historical control range, the NOAEL for these effects was 20 mg/kg bw per day.

In a second study, HY/CR New Zealand White rabbits were given folpet at a dose of 0, 10, 40, or 160 mg/kg bw per day on days 7 to 19 of gestation. The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of reductions in body-weight gain and in gravid uterine weight. The NOAEL for fetal toxicity was 10 mg/kg bw per day on the basis of an increased incidence of bilateral lumbar ribs and delayed skeletal maturation.

In a pulse-dose study, pregnant D1A Hra:(NZW) rabbits were given folpet at a dose of 60 mg/kg bw per day by gavage on days 7 to 9, 10 to 12, 13 to 15, or 16 to 18 of gestation. There were occasional occurrences of abortion, but it was not clear whether these abortions were related to treatment with folpet. Maternal body weight and food consumption were significantly reduced in all treated animals. Two fetuses with hydrocephalus were observed, one in the group treated on days 10 to 12 of gestation and one in the group treated on days 16 to 18 of gestation. These incidences were considered to be within the historical control range. A significantly increased incidence (12.1%) of fetuses with an irregularly shaped fontanelle was observed in the group treated on days 13 to 15 of gestation; the incidence in controls was 4.5%. The significance of these effects was not clear.

Toxicological evaluation

Other than developmental effects, folpet produced no toxicological effects that might be considered to be a consequence of acute exposure. The Meeting concluded that it was not necessary to establish an ARfD for the general population, including children aged 1–6 years for whom separate data on dietary intake are available. The Meeting concluded that it might be necessary to establish an ARfD to protect the embryo or fetus from possible effects in utero. Such an ARfD would apply to women of childbearing age.

The maternal toxicity and the associated reductions in fetal body weight, delayed ossification and increased incidences in skeletal variations observed in studies of developmental toxicity in rabbits are likely to be caused by high local concentrations of folpet and are not considered to be relevant to dietary exposure. However, the increased incidence of hydrocephalus observed could not be attributed with confidence to maternal toxicity.

The Meeting concluded that the database was insufficient (in particular, with regard to the absence of studies on the developmental effects of phthalimide) to establish the mode of action by which the increased incidence of hydrocephalus, observed in rabbits at 60 mg/kg bw per day (NOAEL, 20 mg/kg bw per day) was induced, and as a consequence, their relevance for deriving an ARfD could not be dismissed. Therefore the Meeting established an ARfD of 0.2 mg/kg bw based on a NOAEL of 20 mg/kg bw per day for the increased incidence of hydrocephalus at 60 mg/kg bw per day in rabbits and a safety factor of 100. The use of a safety factor of 100 was considered to be conservative; although the mode of action by which the developmental effects are induced was uncertain, they are possibly secondary to maternal toxicity. The Meeting noted that it might be possible to refine this ARfD using the results of an appropriately designed study.

Estimate of acute reference dose

0.2 mg/kg bw for women of childbearing age

Unnecessary for the general population

References

Christian, M.S. & Hoberman, A.M. (1983a) Pilot teratology study in rats with folpet technical. Unpublished report from Argus Research Laboratories Inc. Evaluated in Annex 1, reference 43.

Christian, M.S. & Hoberman, A.M. (1983b) Teratology study in rats with folpet technical. Unpublished report No. 303-001 from Argus Research Laboratories, Inc. Submitted to WHO by Makhteshim Chemical Works Ltd, Beer-Sheva, Israel.

Feussner, E.L., Hoberman, A.M., Johnson, E.M. & Christian, M.S. (1984) Teratology study in rabbit with folpet technical. Unpublished report No. 303-002 from Argus Research Laboratories, Inc. Submitted to WHO by Makhteshim Chemical Works Ltd, Beer-Sheva, Israel.

Feussner, E.L., Hoberman, A.M., Johnson, E.M. & Christian, M.S. (1985) Teratology study in rabbits with folpet technical using a "pulse-dosing" regimen. Unpublished report No. 303-004 from Argus Research Laboratories, Inc., Horsham, PA, USA. Submitted to WHO by Makhteshim Chemical Works Ltd, Beer-Sheva, Israel.

Gordon, E.B., Ehrlich, T., Mobley, S. & Williams, M. (2001) Measurement of the reaction between the fungicides captan or folpet and blood thiols. *Toxicol. Methods*, **11**, 209–223.

Moore, G.E. (2004) Intestinal irritation in CD-1 mice after a 24-hour exposure to folpet. Unpublished report No. 13763 from Product Safety Laboratory, Dayton, USA. Submitted to WHO by Makhteshim Chemical Works Ltd, Beer-Sheva, Israel.

Myers, D.P. (2003) Folpet. Study of effects on embryo-fetal development in CD rats treated by oral gavage administration. Unpublished report No. MAK 767/023430 from Huntingdon Life Sciences, Huntingdon, UK. Submitted to WHO by Makhteshim Chemical Works Ltd, Beer-Sheva, Israel.

Robens, J.F. (1970) Teratogenic activity of several phthalimide derivates in the golden hamster. *Toxicol. Appl. Pharmacol.*, **16**, 24–34.

Rubin, Y. & Nyska, A. (1985a) Folpan. Teratology study in the rat. Unpublished report No. MAK/049/FOL from Life Science Research Israel Ltd., Ness Ziona, Israel. Evaluated in Annex 1, reference 49.

Rubin, Y. & Nyska, A. (1985b) Folpan. Preliminary teratology study in rabbits. Unpublished report No. MAK/050/FOL from Life Science Research Israel Ltd., Ness Ziona, Israel. Submitted to WHO by Makhteshim Chemical Works Ltd, Beer-Sheva, Israel.

Rubin, Y. (1985) Folpan. Teratology study in the rabbit. Unpublished report No. MAK/051/FOL from Life Science Research Israel Ltd., Ness Ziona, Israel. Submitted to WHO by Makhteshim Chemical Works Ltd, Beer-Sheva, Israel.

GLYPHOSATE

First draft prepared by
Rudolf Pfeil and Lars Niemann
Federal Institute for Risk Assessment, Berlin, Germany

Explanation

Glyphosate (*N*-(phosphonomethyl)glycine) is a non-selective systemic herbicide that was last evaluated by the JMPR in 1986, when an acceptable daily intake (ADI) of 0–0.3 mg/kg bw was established based on a no-observed-adverse-effect level (NOAEL) of 31 mg/kg bw per day, the highest dose tested in a 26-month study of toxicity in rats. In 1997, the Joint Meeting evaluated aminomethylphosphonic acid (AMPA), the major metabolite of glyphosate, and concluded that AMPA was of no greater toxicological concern than its parent compound. A group ADI of 0–0.3 mg/kg bw was established for AMPA alone or in combination with glyphosate. Glyphosate was re-evaluated by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues. The Meeting reviewed new data on glyphosate that had not been reviewed previously and relevant data from the previous evaluations.

Evaluation for acceptable daily intake

Several of the studies performed with glyphosate or AMPA were finalized before the OECD guidelines for testing of chemicals and the regulations for good laboratory practice were enacted. Nevertheless, all the relevant studies were subjected to quality assurance and, with few exceptions, their protocols complied with present guideline requirements.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The absorption, distribution and excretion of glyphosate has been studied in a number of animal species (rats, rabbits, monkey, goats, chickens) treated with single or repeated doses (6.7–1000 mg/kg bw) and by different routes of application (oral, intramuscular, intraperitoneal, intravenous). The results of studies relating to urinary and faecal excretion and residues in tissues are summarized in Table 1.

Rats

Concentrations of radiolabel in the plasma and bone marrow were studied in nine male and nine female Crl:CD BR rats given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 98.7%; radiochemical purity, 98%) as a single intraperitoneal dose at 1150 mg/kg bw. The rats were housed individually in metabolism cages and blood samples were collected from three to six rats after 0.25, 0.5, 1, 2, 4, 6 and 10 h. At

Table 1. Excretion and residues of radioactivity after oral or parenteral administration of ^{14}C-labelled glyphosate, expressed as a percentage of the administered dose

Dose	Species	Urine		Faeces		Tissues		Reference
		Males	Females	Males	Females	Males	Females	
Single dose, oral administration								
6.7 mg/kg bw, 120 h[a]	Rat	14–16	35–43	81–85	49–55	0.14–0.65	0.83–1.02	Colvin & Miller (1973a)[d]
10 mg/kg bw, 24/48 h	Rat	17.9/34.0	12.8/12.5	59.3/60.5	80.3/91.2	ND	ND	Davies (1996d)
10 mg/kg bw, 72 h	Rat	13.0	10.6	88.5	88.7	0.59	0.49	Davies (1996a)
10 mg/kg bw, 168 h	Rat	28.6	22.5	62.4	69.4	0.44	0.31	Ridley & Mirly (1988)
30 mg/kg bw, 168 h	Rat	29.04	30.71	58.84	56.53	0.62	0.64	Powles (1992b)
1000 mg/kg bw, 72 h	Rat	16.7	17.5	89.6	84.5	0.52	0.58	Davies (1996b)
1000 mg/kg bw, 168 h	Rat	30.55	22.41	53.27	60.37	0.47	0.40	Powles (1992b)
1000 mg/kg bw, 168 h	Rat	17.8	14.3	68.9	69.4	0.28	0.24	Ridley & Mirly (1988)
5.7–8.8 mg/kg bw, 120 h	Rabbit	7–11	ND	80–97	ND	0.1–1.2	ND	Colvin & Miller (1973b)[d]
Single dose, intraperitoneal administration								
2.3–3.6 mg/kg bw, 120 h	Rat	82–90	ND	6–14	ND	0.53–1.00	ND	Colvin & Miller (1973a)[d]
Single dose, intravenous administration								
10 mg/kg bw, 168 h	Rat	79.0	74.5	4.65	8.3	1.27	1.09	Ridley & Mirly (1988)
30 mg/kg bw, 168 h	Rat	85.98	84.18	3.42	1.48	1.35	1.09	Powles (1992b)
Single dose, intramuscular administration								
4 mg/animal, 168 h	Monkey	89.9	ND	ND	ND	ND	ND	Maibach (1983)
Repeated doses, oral administration								
10 mg/kg bw, 72 h[b]	Rat	10.6	10.7	86.6	90.7	0.46	0.41	Davies (1996c)
10 mg/kg bw, 168 h[b]	Rat	30.9	23.1	61.0	70.9	0.54	0.35	Ridley & Mirly (1988)
30 mg/kg bw, 168 h[b]	Rat	34.28	34.63	49.64	46.73	0.96	0.83	Powles (1992b)
400 mg/animal per day, 120 h	Goat	ND	9.44	ND	78.16	ND	ND	Powles (1994a)
30 mg/hen per day, 168 h	Hen	ND	ND	ND	76.45c	ND	ND	Powles (1994b)

IM, intramuscular; IP, intraperitoneal; IV, intravenous; ND, not determined

[a] Glyphosate labelled with ^{14}C at the methylene carbon, at the C1-glycine carbon or at the C2-glycine carbon.

[b] 14 daily doses of unlabelled glyphosate at 10 mg/kg bw, followed by a single dose of ^{14}C-labelled glyphosate at 10 mg/kg bw.

[c] Reported as excreta

[d] Cited in Annex 1, reference 47

0.5, 4 and 10 h after dosing, three animals of each sex were killed and the femoral bone marrow was isolated. The plasma and bone marrow samples were analysed for radioactivity by liquid scintillation counting.

Peak levels of radioactivity were observed at 0.5 h after dosing in plasma (males, 1867 mg/kg; females, 2019 mg/kg) and bone marrow (males, 267 mg/kg; females, 413 mg/kg). The amount of radioactivity in the plasma decreased rapidly, while it remained more constant in bone marrow over the experimental period of 10 h. The analysis of the first order elimination rates indicated a half-life time of 1 h (males and females) for plasma and 4.2 h (females) or 7.6 h (males) for bone marrow (Ridley, 1983).

In a study of absorption, distribution and excretion which was considered concisely by the 1997 JMPR for the evaluation of AMPA, groups of five male and five female Crl:CD(SD)BR rats received [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, >99.8%; radiochemical purity, >99%) orally by gavage as a single dose at 10 or 1000 mg/kg bw, or intravenously at a single dose at 10 mg/kg bw. A further group of five male and five female rats received unlabelled glyphosate as 14 consecutive oral doses at 10 mg/kg bw per day followed by ^{14}C-labelled glyphosate as a single oral dose at 10 mg/kg bw. For measurement of radioactivity in expired air, an additional test group of three male and three female rats received a single oral dose at 10 mg/kg bw, and expired gases were removed at 6, 12 and 24 h after dosing. For determination of pharmacokinetic parameters, additional test groups of three male and three female rats received a single dose at 10 mg/kg bw orally or intravenously, and blood samples were taken from the tail vein at various times between 0.25 and 168 h after dosing. The animals were housed individually in metabolism cages from which urine and faeces were collected at regular intervals. Animals used for detection of radioactivity in expired air were sacrificed at 24 h and all remaining animals at 7 days after dosing, and selected tissues were removed. Radioactivity in urine, faeces, blood, expired air and tissues was determined by liquid scintillation counting.

After a single oral dose of 10 mg/kg bw, <0.2% of the administered radioactivity was found in the expired air at 24 h after dosing, and therefore expired gases were not collected for the other test groups.

After a single intravenous dose at 10 mg/kg bw, 74.5–79.0% of the administered dose was eliminated in urine and 4.7–8.3% in the faeces (Table 2). Less than 0.1% of the administered dose was found in the organs taken at necropsy, with approximately 1% of the administered dose remaining in the residual carcass.

For the groups treated orally, most of the administered dose was eliminated in the faeces at both 10 mg/kg bw (62.4–69.4%) and 1000 mg/kg bw (68.9–69.4%), with the urine accounting for 22.5–28.6% and 14.3–17.8% of the administered dose at the lower and higher doses, respectively (Table 1.1-6). Less than 0.05% of the administered dose appeared in the organs after oral dosing and <0.5% remained in the residual carcass. Repeated dosing at 10 mg/kg bw had no significant effect on the routes of excretion of ^{14}C-labelled glyphosate nor on the percentage of the administered dose remaining in the organs, tissues and residual carcass at sacrifice.

Analysis of individual tissues demonstrated that bone contained the highest concentration of [^{14}C]glyphosate equivalents (0.3–31 ppm). The remaining tissues contained

Table 2. Recovery of radioactivity (% of administered dose) in excreta and tissues from rats given ^{14}C-labelled glyphosate

Excreta/tissue	Dose (mg/kg bw)							
	10 (single dose, intravenous)		10 (single dose, oral)		10 (repeated doses, oral)		1000 (single dose, oral)	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine	79.0	74.5	28.6	22.5	30.9	23.1	17.8	14.3
Faeces	4.65	8.30	62.4	69.4	61.0	70.9	68.9	69.4
Organs/tissues	0.0941	0.0521	0.0460	0.0194	0.0473	0.0313	0.0355	0.0266
Residual carcass	1.18	1.04	0.395	0.286	0.497	0.315	0.248	0.208
Gastrointestinal tract contents	0.0394	0.0388	0.0226	0.0145	0.0138	0.0095	0.0258	0.0429
Cage wash	0.890	1.30	1.30	1.96	0.820	1.96	3.86	8.00
Total recovery[a]	86.0	85.3	92.8	94.2	93.3	96.3	90.9	92.1

From Ridley & Mirly (1988)
[a] Total recovery is the mean of values for individual animals

Table 3. Mean tissue concentration of radioactivity (ppm) at 168 h in rats given ^{14}C-labelled glyphosate as single or repeated doses

Tissue	Dose (mg/kg bw)							
	10 (single dose, intravenous)		10 (single dose, oral)		10 (repeated doses, oral)		1000 (single doses, oral)	
	Males	Females	Males	Females	Males	Females	Males	Females
Whole blood	0.0185	0.00996	0.00454	0.00269	0.00476	0.00288	0.328	0.166
Liver	0.104	0.0498	0.0298	0.0135	0.0407	0.0257	1.91	1.37
Brain	0.0414	0.0360	0.00705	0.00551	0.0144	0.0110	0.750	0.556
Kidney	0.106	0.0714	0.0216	0.0132	0.0327	0.0196	1.94	1.35
Spleen	0.0439	0.0320	0.0119	0.00727	0.0155	0.0130	2.61	2.98
Lung	0.103	0.0785	0.0148	0.0120	0.0211	0.0167	1.54	1.13
Heart	0.0263	0.0170	0.00622	0.00398	0.00804	0.00632	0.590	0.518
Testes/ovaries	0.0182	0.0223	0.00276	0.00326	0.00529	0.00813	0.363	0.572
Stomach	0.0237	0.0182	0.00795	0.00367	0.0377	0.0239	2.38	2.36
Small intestine	0.0262	0.0164	0.0216	0.0183	0.0441	0.0257	1.90	1.55
Colon	0.0348	0.0178	0.0342	0.0159	0.0429	0.0298	11.0	9.2
Bone	1.48	1.59	0.552	0.313	0.748	0.462	30.6	19.7
Bone marrow	0.0692	0.0303	0.0290	0.00639	0.0245	0.0231	4.1	12.5
Abdominal muscle	0.00766	0.00605	0.00232	0.0016	0.00278	0.00216	0.262	0.214
Shoulder muscle	0.0106	0.0327	0.00388	0.00667	0.00783	0.00590	0.419	0.423
Abdominal fat	0.00535	0.00366	0.00364	0.00324	0.00557	0.00576	0.418	0.457
Residual carcass	0.344	0.337	0.106	0.087	0.157	0.101	8.27	7.74
Tail	0.699	0.611	ND	ND	ND	ND	ND	ND

From Ridley & Mirly (1988)
ND, not determined

glyphosate equivalents at a concentration of between 0.0003 and 11 ppm (Table 3). In the bone and some highly perfused tissues, levels were statistically higher in males than in females.

The estimated half-life for whole body elimination of radioactivity was 2.11–7.52 h for the alpha phase and 69–337 h for the beta phase. The half-life in males at the higher dose was found to be significantly longer than those given the lower dose. Pre-treatment at the lower dose had no significant effect on the whole body elimination.

Based on the area under the curve for the blood concentration of radioactivity after oral or intravenous administration, the oral absorption of glyphosate was found to be 30.3–35.4%. This compared favorably with the absorption (30.2–36.2%) calculated from the data on excretion in the urine after oral and intravenous administration. The results of this study demonstrate that glyphosate is poorly absorbed and rapidly eliminated after a single oral dose at 10 or 1000 mg/kg bw (Ridley & Mirly, 1988).

In a preliminary study of absorption and distribution, male Sprague-Dawley rats were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 98.6%; radiochemical purity, 94.3–97.4%) as a single oral dose at 30 mg/kg bw orally by gavage in 0.9% saline. Blood samples were taken from the tail vein of three animals at various times between 0.5 and 48 h after dosing. Additional animals were killed 4, 10 and 24 h after dosing and the tissue distribution of radioactivity was investigated by whole body autoradiography.

The plasma concentrations of radioactivity reached a maximum 3–4 h after dosing and were in the range of 0.705 to 1.769 µg equivalents/ml. Thereafter the concentration declined rapidly and radioactivity could not be detected 12 h after dosing. The elimination half-life and area under the plasma concentration–time curve were 6.2–12.35 h and 18.62–23.09 µg equivalents.h/ml in two of the rats. Pharmacokinetic parameters could not be calculated for the third animal. Autoradiography showed that the highest concentration of radioactivity was present at 10 h in bone, bone marrow, cartilage, parts of the gastrointestinal tract, kidney, urinary tract and nasal mucosa. At termination 24 h after dosing, the concentation of radioactivity was negligible in all tissues except bone, bone marrow, parts of the gastrointestinal tract, bladder and kidney cortex (Powles, 1992a).

In a study of absorption, distribution and excretion, groups of five male and five female Sprague-Dawley rats were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 96.8%; radiochemical purity, >98%) as a single dose at 30 or 1000 mg/kg bw orally by gavage in saline, or intravenously as a single dose at 30 mg/kg bw. A further group of five male and five female rats received unlabelled glyphosate as 14 consecutive oral doses at 30 mg/kg bw per day followed by ^{14}C-labelled glyphosate as a single oral dose at 30 mg/kg bw. The animals were housed individually in metabolism cages from which urine, faeces and expired air were collected at regular intervals. Animals were sacrificed after 90% of the dose had been eliminated or 7 days after dosing, whichever was sooner. At necropsy, a blood sample was taken and selected tissues were removed. Radioactivity in urine, faeces, blood, expired air and tissues was determined by liquid scintillation counting.

After administration of a single intravenous dose at 30 mg/kg bw, >84% of the dose was eliminated in the urine (Table 4), mostly within 8 h after dosing (Tables 5 and 6). Faecal elimination accounted for <3.5% of the administered radioactivity. Only a very small proportion of the radioactivity was eliminated in exhaled air and <1.4% was present in tissues and the residual carcass when the animals were sacrificed.

In contrast, faeces was the major route of elimination when ^{14}C-labelled glyphosate was given by the oral route. About 57–59% of a single oral dose of 30 mg/kg bw was excreted in the faeces (Table 4); most of this was eliminated in the 12–36 h after dosing (Tables 5 and 6). Urinary elimination was slower for the oral dose at 30 mg/kg bw than for the intravenous dose; 29–31% was eliminated, mainly within 36 h of dosing.

Table 4. Recovery of radioactivity (% of administered dose) in excreta and tissues from rats given ^{14}C-labelled glyphosate

Excreta/tissue	Dose (mg/kg bw)							
	30 (single dose, intravenous)		30 (single dose, oral)		30 (repeated doses, oral)		1000 (single dose, oral)	
	Males	Females	Males	Females	Males	Females	Males	Females
Urine	85.98	84.18	29.04	30.71	34.28	34.63	30.55	22.41
Faeces	3.422	1.484	58.84	56.53	49.64	46.73	53.27	60.37
CO_2	0.024	0.023	0.075	0.065	0.085	0.055	0.064	0.067
Tissues	1.353	1.093	0.619	0.635	0.955	0.825	0.469	0.400
Total[a]	97.75	100.0	96.63	96.71	90.14	89.95	99.70	100.4

From Powles (1992b)
[a] Including cage wash and debris

Table 5. Excretion of radioactivity in the urine and faeces (% of administered dose) by male rats given ^{14}C-labelled glyphosate

Time (h)	Dose (mg/kg bw)							
	30 (single dose, intravenous)		30 (single dose, oral)		30 (repeated doses, oral)		1000 (single dose, oral)	
	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces
0–4	72.10	NS	3.215	NS	8.992	NS	5.561	NS
4–8	7.344	NS	9.638	NS	11.79	NS	14.21	NS
8–12	2.422	1.193	4.239	NS	4.950	24.61	2.918	NS
12–24	1.694	1.035	7.137	45.84	5.091	15.58	3.819	38.63
24–36	0.813	0.530	3.163	8.304	2.387	7.404	1.991	9.788
36–48	0.369	0.258	0.752	3.126	0.622	1.046	0.806	2.385
48–72	0.458	0.191	0.588	1.409	0.447	1.007	0.687	1.612
72–96	0.320	0.158	0.197	0.121	NS	NS	0.247	0.549
96–120	0.187	0.073	0.115	0.040	NS	NS	0.172	0.081
120–144	0.141	0.023	NS	NS	NS	NS	0.096	0.165
144–168	0.112	0.029	NS	NS	NS	NS	0.043	0.058
Total	85.98	3.422	29.04	58.84	34.28	49.64	30.55	53.27

From Powles (1992b)
NS, no sample, either because no faeces were voided during the collection period or because collection had ceased before that time-point.

Excretion was unaffected by administration of unlabelled glyphosate for 14 days prior to the administration of ^{14}C-labelled glyphosate and the routes and rates of excretion of a higher dose of ^{14}C-labelled glyphosate (1000 mg/kg bw) were almost identical to those at the lower dose (Tables 4, 5, 6). There was no significant sex difference in the elimination of glyphosate for any dose regimen. Irrespective of the dose, route or frequency of duration <1.4% of the administered dose was retained in tissues. The highest concentration of radioactivity was present in bone, with lower concentrations in bone marrow, kidney, liver, lungs and the residual carcass (Table 7) (Powles, 1992b).

In a study of absorption, distribution and excretion, groups of five male and five female Alpk:AP$_f$SD rats were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 99.2–99.5%; radiochemical purity, >98%) as a single dose at 10 or 1000 mg/kg bw orally by gavage in deionized water. An additional group of five male

Table 6. Excretion of radioactivity in the urine and faeces (% of administered dose) by female rats given ^{14}C-labelled glyphosate

Time (h)	Dose (mg/kg bw)							
	30 (single dose, intravenous)		30 (single dose, oral)		30 (repeated doses, oral)		1000 (single dose, oral)	
	Urine	Faeces	Urine	Faeces	Urine	Faeces	Urine	Faeces
0–4	71.89	NS	3.150	NS	5.515	NS	2.078	NS
4–8	6.397	NS	11.91	NS	15.21	NS	13.32	NS
8–12	2.056	0.502	4.489	NS	5.006	17.44	2.386	NS
12–24	1.429	0.631	7.202	40.68	6.184	18.25	2.622	48.27
24–36	0.778	0.145[a]	2.266	10.94	1.520	8.613	0.966	8.524
36–48	0.364	0.388	0.775	2.455	0.427	1.498	0.378	1.945
48–72	0.497	0.178	0.551	2.158	0.766	0.925	0.384	1.086
72–96	0.309	0.047	0.238	0.235	NS	NS	0.165	0.505
96–120	0.206	0.020	0.134	0.059	NS	NS	0.096	0.029
120–144	0.145	0.000	NS	NS	NS	NS	0.012	0.006
144–168	0.109	0.000	NS	NS	NS	NS	0.000	0.009
Total	84.18	1.484	30.71	56.53	34.63	46.73	22.41	60.37

From Powles (1992b)
NS, no sample, either because no faeces were voided during the collection period or because collection had ceased before that time-point.
[a] Only one sample was analysed for this time-point.

Table 7. Mean tissue concentration of radioactivity (ppm) at 168 h in rats given ^{14}C-labelled glyphosate as a single dose or as repeated doses

Tissue	Dose (mg/kg bw)							
	30 (single dose, intravenous)		30 (single dose, oral)		30 (repeated doses, oral)		1000 (single dose, oral)	
	Males	Females	Males	Females	Males	Females	Males	Females
Blood	0.050	0.084	0.011	0.000	0.000	0.000	0.000	0.000
Bone	4.195	4.355	2.246	2.562	3.096	2.505	56.32	40.66
Bone marrow	0.255	1.264	0.322	0.545	0.325	0.144	3.080	0.000
Brain	0.118	0.120	0.056	0.056	0.019	0.000	0.000	0.000
Abdominal fat	0.000	0.000	0.000	0.009	0.000	0.000	0.000	0.000
Carcass	0.423	0.335	0.197	0.214	0.339	0.284	5.628	4.476
Heart	0.051	0.025	0.051	0.045	0.000	0.000	0.000	0.000
Kidney	0.304	0.298	0.278	0.205	0.515	0.317	5.170	3.968
Liver	0.241	0.222	0.251	0.254	0.615	0.425	6.144	0.000
Lungs	0.264	0.279	0.124	0.126	0.183	0.173	2.904	1.216
Muscle	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
Ovaries	NA	0.034	NA	0.068	NA	0.028	NA	0.000
Plasma	0.000	0.000	0.000	0.003	0.000	0.000	0.000	0.000
Salivary gland	0.082	0.068	0.053	0.079	0.084	0.100	0.000	0.000
Spleen	0.117	0.117	0.140	0.091	0.164	0.153	0.000	0.000
Testes	0.000	NA	0.000	NA	0.000	NA	0.000	NA
Uterus	NA	0.248	NA	0.143	NA	0.239	NA	0.000

From Powles (1992b)
NA, not applicable

and five female rats received unlabelled glyphosate as consecutive oral doses at 10 mg/kg bw per day for 14 days followed by ^{14}C-labelled glyphosate as a single oral dose at 10 mg/kg bw. The animals were housed individually in metabolism cages from which urine and faeces were collected at regular intervals. At termination 72 h after dosing, representative samples

of tissues were removed. Radioactivity in urine, faeces and tissues was determined by liquid scintillation counting.

After a single oral dose, the rate of excretion was rapid, with >87% of the dose being excreted within 24 h of dosing by both sexes, and total excretion was effectively complete after 72 h. No pronounced sex difference was apparent in either the routes or rates of excretion. Faeces was the predominant route of excretion, accounting for 88.5–89.6% and 84.5–88.7% of the administered dose in males and females, respectively, while excretion via urine accounted for 13.0–16.6% and 10.6–17.5% in males and females, respectively. At termination, tissue concentration of radioactivity was very low and accounted for <0.6% of the administered dose in both sexes (Table 8). The highest concentrations were present in bone (Table 9).

After repeated dosing, the rate of excretion was rapid, with >90% of the administered dose being excreted in both sexes within 24 h of dosing, and total excretion was effectively complete after 72 h. No pronounced sex difference was apparent in either the routes or rates of excretion. Faeces was the predominant route of excretion, accounting for 86.6% and 90.7% of the administered dose in males and females, respectively, while excretion via urine accounted for 10.6% and 10.7% in males and females, respectively. At termination, the tissue concentration of radioactivity was very low and accounted for <0.5% of the administered dose in both sexes (Table 8). The highest concentrations were present in bone (Table 9) (Davies, 1996a, 1996b, 1996c).

In a study of absorption, distribution and excretion, groups of two male and two female Alpk:AP$_f$SD rats received [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 99.2%; radiochemical purity, >98%) orally by gavage in deionized water at a single dose of 10 mg/kg bw. The animals were housed individually in metabolism cages from which urine and faeces were collected at regular intervals. The study was terminated 24 h or 48 h after dosing. Radioactivity in urine and faeces was determined by liquid scintillation counting, and in tissues by whole body autoradiography.

The rate of excretion was rapid, with >77% of the dose excreted within 24 h of dosing by both sexes, and total excretion was practically complete after 48 h. Faeces was the predominant route of excretion, accounting for 59.3–60.5% and 80.3–91.2% in males and females, respectively, while excretion via urine accounted for 17.9–34.0% and 12.5–12.8%

Table 8. Recovery of radioactivity (% of administered dose) in excreta and tissues from rats given ^{14}C-labelled glyphosate

Excreta/tissue	Dose (mg/kg bw)					
	10 (single dose)		10 (repeated doses)		1000 (single dose)	
	Males	Females	Males	Females	Males	Females
Urine	13.0	10.6	10.6	10.7	16.7	17.5
Faeces	88.5	88.7	86.6	90.7	89.6	84.5
Gastrointestinal tract and contents	0.19	0.17	0.1	0.1	0.2	0.22
Cage wash	0.3	0.4	0.2	0.2	0.1	0.2
Tissues (including carcass)	0.59	0.49	0.46	0.41	0.52	0.58
Total	102.6	100.3	98.0	102.2	107.1	103.1

From Davies (1996a, 1996b, 1996c)

Table 9. Mean tissue concentration of radioactivity (ppm) at 72 h in rats given ^{14}C-labelled glyphosate as a single oral dose or as repeated oral doses

Tissue	Dose (mg/kg bw)					
	10 (single dose)		10 (repeated doses)		1000 (single dose)	
	Males	Females	Males	Females	Males	Females
Brain	0.011	0.009	0.010	0.010	1.23	1.16
Gonads	0.007	0.024	0.007	0.026	0.91	2.94
Heart	0.012	0.011	0.011	0.012	1.11	1.25
Kidneys	0.068	0.049	0.061	0.049	6.51	6.05
Liver	0.059	0.044	0.055	0.045	5.48	5.23
Lungs	0.031	0.026	0.026	0.029	2.87	3.54
Spleen	0.026	0.024	0.022	0.025	2.44	3.11
Salivary glands	0.017	0.018	0.019	0.027	1.81	2.09
Abdominal fat	0.007	<0.004	0.008	0.006	0.54	0.50
Bone (femur)	0.511	0.395	0.358	0.345	49.78	44.93
Muscle	0.007	0.006	0.008	0.007	0.82	0.83
Blood	0.011	0.009	0.014	0.010	0.89	0.80
Plasma	<0.004	<0.004	<0.004	<0.005	<0.40	<0.40
Residual carcass	0.062	0.056	0.050	0.046	4.77	5.86

From Davies (1996a, 1996b, 1996c)
Limit of detection was 0.004 or 0.35 µg equivalents/g (at 10 or 1000 mg of ^{14}C-labelled glyphosate/kg bw, respectively)

in males and females, respectively. At termination, the greatest intensity of tissue radioactivity was present in bone at 24 h and 48 h. Some radioactivity was also apparent in the kidneys after 24 h, but had declined to negligible amounts after 48 h (Davies, 1996d).

Goats

In a study of absorption, distribution, metabolism and excretion, two lactating goats (strain, British Saanen; age, approximately 3 years of age; body weight, approximately 46.5 and 62 kg) were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 97.5%; radiochemical purity, >97%) as repeated doses at a mean dose of 355 or 400 mg/animal per day (equivalent to a nominal dietary concentration of 200 ppm) by oral gavage for 5 or 3 consecutive days. Excreta were collected from both animals at 24-h intervals after the first dose. The goats were milked twice daily and the milk was pooled to provide a daily sample for each animal. The first goat, which was given five consecutive doses, was killed approximately 23.5 h after the last dose, and the liver, kidneys and samples of muscle and fat were removed at necropsy. From the second goat, given three consecutive doses, blood samples were taken at 1, 2, 3, 4, 6, 8, 12 and 24 h after the initial dose. The second goat was killed when the plasma concentration of radiolabel was at a maximum (approximately 8 h after the final dose). Excreta, milk and tissues from this animal were used for identification of metabolites.

For the first goat, the overall recovery of administered radioactivity was 89.9%, most of which was present in faeces (78.16%), urine (9.44%) and cage debris/cage wash (2.22%). Negligible radioactivity was recovered from milk (0.03%) and tissues (0.05%). The transfer coefficient for milk was low (approximately 0.07%) with peak concentration (0.072 ppm) achieved on day 4 of dosing. At necropsy, residues were highest in kidney (3.852 ppm), liver (0.404 ppm) and skeletal muscle (0.035 ppm), and below the limit of detection (0.028 ppm) in fat.

For the second goat, at 8 h after the final dose, 57.6% of administered radioactivity was recovered in the excreta, with 52.6, 4.7 and 0.03% present in the faeces, urine and milk, respectively. The concentration of radioactivity in the plasma peaked at 6–8 h after the initial dose (0.102–0.101 ppm), while the concentration in milk was highest on day 3 of dosing (0.086 ppm). At necropsy, residues were highest in kidney (12.15 ppm), liver (0.225 ppm) and skeletal muscle (0.061 ppm), and below the limit of detection (0.036 ppm) in fat. Unchanged glyphosate was the major component detected in both urine and faeces (94–96%) by high-performance liquid chromatography (HPLC) and confirmed by Fourier-transform infrared (FT-IR) spectroscopy. Small amounts of AMPA were tentatively identified in the urine and faeces by thin-layer chromatography (TLC), but not confirmed by HPLC. The main residues in tissues and milk were glyphosate, with only low levels of AMPA tentatively identified in kidney (Powles, 1994a).

Chickens

In a study of absorption, distribution, metabolism and excretion, two groups of five laying hens (strain, ISA; age, 20–22 weeks; body weight, approximately 1.5 kg) were given [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 97.5%; radiochemical purity, >97%) as repeated doses at a mean dose of approximately 30 mg/animal per day (equivalent to a nominal dietary concentration of 200 ppm) by oral gavage for 7 or 5 consecutive days (group A and B, respectively). Excreta and eggs were collected at 24-h intervals after the initial dose. From each animal in group B, blood samples were collected at 1, 2, 3, 4, 6, 8 and 12 h after the first dose. The hens were sacrificed 23.5 h after the last dose (group A) or 1 h after the last dose when the plasma concentration of radioactivity was at a maximum (group B). At necropsy, selected tissues were removed from each bird and radioactivity was determined in all excreta, egg yolk, egg white, liver, muscle, fat and skin samples. Tissues from hens in group B were used for identification of metabolites.

In hens in group A, the overall recovery of administered radioactivity was 80.34% at termination, with 76.45% being present in excreta and 3.86% in cage wash/cage debris, and negligible amounts in tissues (0.02 ppm) and eggs (<0.01 ppm). Radioactivity in egg white reached a plateau (0.059 ppm) at day 6, while the concentration in yolks increased throughout the dosing period and reached 0.484 ppm at day 7. At study termination, radioactivity was detected in liver, skin and fat (1.242, 0.212 and 0.153 ppm, respectively), but was below the limit of detection (0.043 ppm) in muscle.

In hens in group B, the concentration of radioactivity in the plasma attained a maximum (0.475 ppm) 1 h after the initial dose, then declined slowly and was still measurable in one hen 12 h after dosing. At termination, radioactivity was present in liver, skin, fat and muscle (1.080, 0.359, 0.083 and 0.041 ppm, respectively). The residues in egg white and yolk were highest at day 5 (0.072 and 0.228 ppm, respectively). Unchanged glyphosate was the major component detected in excreta, liver, skin, fat, muscle, egg white and egg yolk by TLC (and confirmed by FT-IR spectroscopy). Small amounts of AMPA were detected in excreta, liver and skin by TLC but, in each case, confirmation of the presence of that metabolite could not be confirmed by HPLC (Powles, 1994b).

1.2 Biotransformation

In a study of biotransformation that was considered concisely by the 1997 JMPR for the evaluation of AMPA, groups of five male and five female Crl:CD(SD)BR rats received

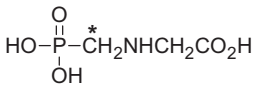
[^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, >99.8%; radiochemical purity, >99%) as a single oral dose at 10 or 1000 mg/kg bw, as repeated oral doses at 10 mg/kg bw per day, or as a single intravenous dose at 10 mg/kg bw (for details of the study design, see Ridley & Mirly, 1988). For identification and quantification of parent compound and metabolites in urine and faecal samples, chromatographic (cation-exchange HPLC, ion-pair HPLC) and spectroscopic (nuclear magnetic resonance [NMR]; mass spectrometry [MS]) techniques were used.

Glyphosate was isolated as the predominant radioactive fraction in the urine (overall recovery, 81.3%) and faeces (overall recovery, 99.2%) and was positively identified in each case by ^{1}H-NMR, ^{13}P-NMR and by MS with and without derivatization. The minimum content of glyphosate in either urine or faecal samples from the individual rats was 97.46%. HPLC analyses further indicated that glyphosate in the excreta accounted for 98.50–99.33% of the administered [^{14}C]glyphosate. In rats dosed orally at 10 mg/kg bw, either as single or multiple doses, there was evidence for formation of 0.2–0.3% and 0.4% AMPA, respectively, from metabolism of glyphosate in vivo. Since AMPA was not formed after intravenous administration, it seems likely that the formation of AMPA after oral administration of glyphostae at low doses is due to a very minor amount of gastrointestinal metabolism of glyphosate, possibly by the gastrointestinal microflora (Howe et al., 1988).

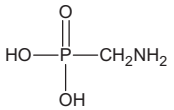
In a study of excretion and biotransformation, two male and two female bile-duct cannulated Alpk: AP$_f$SD rats received [^{14}C]phosphonomethyl-labelled glyphosate (purity of unlabelled test substance, 99.5%; radiochemical purity, 97.8%) as a single dose at 1000 mg/kg bw given orally by gavage. The rats were housed individually in metabolism cages, and urine, faeces and bile were collected at regular intervals between 2 h and 48 h after dosing. The samples were analysed for radioactivity by liquid scintillation counting. For identification and quantification of parent compound and metabolites in urine and faecal samples from previous studies performed by Davies (1996a, 1996b, 1996c), chromatographic (TLC, HPLC) and spectroscopic (NMR) techniques were used.

For male and female bile-duct cannulated rats, excretion of administered radioactivity over 48 h was 20.8% and 16.3 % in the urine, 39.1% and 30.5% in the faeces, and 0.06% and 0.06% in bile, respectively. In faecal samples from previous studies, it was confirmed that all extracted radioactivity was glyphosate. In urine samples, the major radioactive component was unchanged glyphosate (10.5–16.7% of administered dose), while only

Figure 1. Structural formulae of glyphosate and AMPA



Glyphosate (* Denotes position of radiolabel)



Aminomethylphosphonic acid (AMPA)

trace amounts of aminomethyl phosphonic acid (AMPA, 0.07–0.66%) were detected (Macpherson, 1996).

2. Toxicological studies

2.1 Acute toxicity

(a) Lethal doses

The results of tests for the acute oral, dermal or inhalation toxicity of glyphosate are summarized in Table 10.

Groups of 10 ICR mice of each sex were given glyphosate as a single dose at 1000, 5000 or 10 000 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. Two out of 10 males and one out of 10 females at 10 000 mg/kg bw died. Reduced activity was observed at 5000 mg/kg bw and greater. No treatment-related gross necropsy changes were found at sacrifice (Shirasu & Takahashi, 1975).

Groups of five male and five female Bom:NMRI mice were given glyphosate as a single dose at 2000 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. All animals survived until study termination. Treatment-related clinical signs were observed in all mice on day 1 and included piloerection and sedation. No treatment-related gross necropsy changes were found at sacrifice (Dideriksen, 1991).

Groups of five male and five female fasted Wistar rats were given glyphosate as a single dose at 2500, 3500, 5000, 7000 or 9900 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. Mortality occurred in 1 out of 10, 1 out of 10, 3 out of 10, 8

Table 10. Acute toxicity of glyphosate

Species	Strain	Sex	Route	Vehicle (particle size)	Purity (%)	LD_{50} (mg/kg bw) LC_{50} (mg/l)	Reference
Mouse	ICR	10F, 10M per dose	Oral	0.2% carboxy-methylcellulose	96.7	>10 000	Shirasu & Takahashi (1975)
Mouse	NMRI	5F, 5M	Oral	Water	98.6	>2 000	Dideriksen (1991)
Rat	Wistar	5F, 5M per dose	Oral	Water	99.0	5 600 (4 900–6 300)	Heenehan (1979a)
Rat	Sprague-Dawley	5F, 5M	Oral	1% Methocel	85.5	>5 000	Blaszcak (1988a)
Rat	Sprague-Dawley	5F, 5M	Oral	Water	97.76	>5 000	Reagan (1988a)
Rat	Sprague-Dawley	5F, 5M	Oral	0.5% carboxy-methylcellulose	98.6	>5 000	Cuthbert & Jackson (1989a)
Rat	Wistar	5F, 5M	Oral	Water	95.6	>5 000	Doyle (1996a)
Goat	(Spanish)	5F	Oral	Water	98.7	3 530 (2 950–4 220)	Rowe et al. (1987)
Rat	Wistar	5F, 5M	Dermal	Water	95.6	>2 000	Doyle (1996b)
Rabbit	New Zealand White	2F, 2M	Dermal	0.9% saline	99	>5 000	Heenehan (1979b)
Rabbit	New Zealand White	5F, 5M	Dermal	0.9% saline	85.5	>5 000	Blaszcak (1988b)
Rabbit	New Zealand White	5F, 5M	Dermal	0.9% saline	97.76	>5 000	Reagan (1988b)
Rat	Sprague-Dawley	5F, 5M	Inhalation (4 h, nose-only)	None (22.5 µm)	98.6	>4.98	McDonald & Anderson (1989)
Rat	Wistar	5F, 5M	Inhalation (4 h, nose-only)	None (2.9–3.6 µm)	95.6	>4.43	Rattray (1996)

F, female; M, male

out of 10 and 10 of 10 rats at 2500, 3500, 5000, 7000 and 9900 mg/kg bw, respectively. Treatment-related clinical signs were observed at all doses and included ataxia, convulsions, muscle tremors, red nasal discharge, clear oral discharge, urinary staining of the abdomen, soft stool, piloerection, lethargy, and faecal staining of the abdomen. Treatment-related gross necropsy changes were usually observed in animals that died and included brown/white fluid in the stomach or the intestine and coloured spots in the lungs, the liver and the kidneys (Heenehan et al., 1979a).

Groups of five male and five female fasted CD (Sprague-Dawley derived) rats were given glyphosate as a single dose at 5000 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. All animals survived until study termination. Treatment-related clinical signs included wet rales, faecal staining, urinary staining and soft stools. Some animals had decreased food consumption after dosing; and one of the five females exhibited weight loss on day 7, but gained weight between days 7 and 14. No treatment-related gross necropsy changes were found at sacrifice (Blaszcak, 1988a).

Groups of five male and five female fasted Sprague-Dawley rats were given glyphosate as a single dose at 5000 mg/kg bw orally by gavage and were observed for 15 days before sacrifice. All animals survived until study termination. Treatment-related clinical signs included diarrhoea, apparent urinary incontinence and hair loss on the abdomen. There were no effects on body weights, and no treatment-related changes were found at gross necropsy after sacrifice (Reagan, 1988a).

Groups of five male and five female fasted Sprague-Dawley rats were given glyphosate as a single dose at 5000 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. All animals survived until study termination. Treatment-related clinical signs included piloerection, reduced activity and ataxia through day 9. No treatment-related gross necropsy changes were found at sacrifice (Cuthbert & Jackson, 1989a).

Groups of five male and five female fasted Alpk:AP_fSD (Wistar-derived) rats were given glyphosate as a single dose at 5000 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. All animals survived until study termination, and there were no treatment-related clinical signs. All animals lost weight initially because they had been fasted before dosing, but all exceeded their initial weight by day 3 and, apart from a transient weight loss in one female, continued to gain weight throughout the remainder of the study. No treatment-related gross necropsy changes were found at sacrifice (Doyle, 1996a).

Groups of five female Spanish goats were given glyphosate as a single dose at 1980, 3090, 4620 or 10 000 mg/kg bw orally by gavage and were observed for 14 days before sacrifice. Mortality was none out of five, one out of five, five out of five and five out of five at 1980, 3090, 4620 or 10 000 mg/kg bw, respectively. Treatment-related clinical signs included diarrhoea (at all doses) and colic, depression, and ataxia at 3090 mg/kg bw and greater. All surviving animals appeared to be normal at the end of the experiment. At gross necropsy, no treatment-related changes were found. Microscopic examination revealed moderate to severe tubular nephrosis in animals that died prior to terminal sacrifice. This lesion may have contributed to the observed elevations in blood urea nitrogen and creatinine (Rowe et al., 1987).

Groups of five male and five female Alpk:AP_fSD (Wistar-derived) rats received glyphosate as a single dermal application at 2000 mg/kg bw and were observed for 14 days

before sacrifice. All animals survived until study termination. There were no treatment-related signs of systemic toxicity and practically no signs of skin irritation, with the exception of one male with slight erythema on days 2 and 3 and one female with scabs from day 3 to 8. Most animals had exceeded their initial weight by the end of the study. No treatment-related gross necropsy changes were found at sacrifice (Doyle, 1996b).

Groups of two male and two female New Zealand White rabbits received glyphosate as a single dermal application at 5000 mg/kg bw and were observed for 14 days before sacrifice. All animals survived until study termination. Very slight erythema was observed in two animals and well-defined erythema was observed in the remaining two animals. Clinical signs included clear nasal discharge in each animal (up to day 6) and weight loss in one animal. No treatment-related gross necropsy changes were found at sacrifice (Heenehan et al., 1979b).

Groups of five male and five female New Zealand White rabbits received glyphosate as a single dermal application at 5000 mg/kg bw and were observed for 14 days before sacrifice. All animals survived until study termination, and no clinical signs or dermal effects were seen throughout the study. All animals exhibited slight body-weight losses or no weight change on day 7, but most had slight weight gains between days 7 and 14. No treatment-related gross necropsy changes were found at sacrifice (Blaszcak, 1988b).

Groups of five male and five female New Zealand White rabbits received glyphosate as a single dermal application at 5000 mg/kg bw and were observed for 14 days before sacrifice. One female rabbit exhibited diarrhoea and/or anorexia on days 9–13 and died on day 14. This finding was considered to be consistent with mucoid enteropathy, a condition occasionally encountered in control rabbits. Therefore, this death was considered to be spontaneous and unrelated to treatment. Anorexia, diarrhoea and soft stools were also noted in two males and one female rabbit that survived to study termination. No treatment-related gross necropsy changes were found at sacrifice (Reagan, 1988b).

Groups of five male and five female Sprague-Dawley rats were exposed to glyphosate at a mean aerial concentration of 4.98 mg/l (mean measured particle size, 22.5 µm) for 4 h in a snout-only system and were observed for 14 days before sacrifice. There was no mortality during the study. Animals were slightly subdued after dosing at day 1 only. There were no effects on body-weight gain, and no treatment-related gross necropsy changes were found at sacrifice (McDonald & Anderson, 1989).

Groups of five male and five female Alpk:AP_fSD (Wistar-derived) rats were exposed to glyphosate at a mean aerial concentration of 2.47 or 4.43 mg/l (mean measured particle size, 2.9–3.6 µm) for 4 h in a nose-only system and were observed for 14 days before sacrifice. At 4.43 mg/l, two out of five males and two out of five females were found dead or were killed in extremis on days 5, 6 or 9 of the study. There was no mortality at 2.47 mg/l. Treatment-related clinical signs during exposure were salivation, irregular breathing and auditory hypoaesthesia, while irregular breathing, reduced righting reflex, shaking and splayed gait were observed in both groups immediately after exposure. At necropsy, the two males found dead had dark lungs. No treatment-related gross necropsy changes were found in the other animals (Rattray, 1996).

(b) Dermal and ocular irritation and dermal sensitization

The results of tests for dermal and ocular irritation and dermal sensitization with glyphosate (glyphosate acid and glyphosate salts) are summarized in Table 11.

The potential of glyphosate acid to irritate the skin was evaluated in five studies in male and/or female New Zealand White rabbits. The studies were performed in compliance with the principles of good laboratory practice (GLP) and according to the test guidelines of the United States Environmental Protection Agency (US EPA) or the OECD (TG 404). In the first study, animals with intact skin and abraded skin were exposed for 24 h and the

Table 11. Irritation and sensitization potential of glyphosate

Species	Strain	Sex	End-point	Form of glyphosate, vehicle	Purity (%)	Result	Reference
Rabbit	New Zealand White	3F, 3M	Skin irritation (24 h)[a]	Glyphosate, water	99	Not irritating	Heenehan (1979c)
Rabbit	New Zealand White	2F, 4M	Skin irritation (4 h)	Glyphosate, 0.9% saline	85.5	Minimally irritating	Blaszcak (1988c)
Rabbit	New Zealand White	3F, 3M	Skin irritation (4 h)	Glyphosate, 0.9% saline	97.76	Not irritating	Reagan (1988c)
Rabbit	New Zealand White	4F, 2M	Skin irritation (4 h)	Glyphosate, water	98.6	Not irritating	Cuthbert & Jackson (1989b)
Rabbit	New Zealand White	6F	Skin irritation (4 h)	Glyphosate, water	95.6	Not irritating	Doyle (1996c)
Rabbit	New Zealand White	6NS (3NS[b])	Eye irritation	Glyphosate, 25% w/v in water	99.0	Severely irritating	Heenehan (1979d)
Rabbit	New Zealand White	3F, 3M	Eye irritation	Glyphosate	85.5	Severely irritating	Blaszcak (1988d)
Rabbit	New Zealand White	6NS	Eye irritation	Glyphosate	97.76	Severely irritating	Reagan (1988d)
Rabbit	New Zealand White	1 (NS)	Eye irritation	Glyphosate	98.6	Severely irritating	Cuthbert & Jackson (1989c)
Rabbit	New Zealand White	3F, 3M (3M[b])	Eye irritation	Glyphosate	98.2	Severely irritating	Kuhn (1996)
Rabbit	New Zealand White	6F	Eye irritation	Glyphosate	95.6	Moderately irritating	Johnson (1997)
Rabbit	New Zealand White	3F, 3M (2F, 1M[b])	Eye irritation	Glyphosate IPA salt	65.0	Not irritating	Branch (1981)
Rabbit	New Zealand White	6NS	Eye irritation	Glyphosate ammonium salt	90.8	Slightly irritating	Busch (1987a)
Rabbit	New Zealand White	6NS	Eye irritation	Glyphosate sodium salt	70.7	Slightly irritating	Busch (1987b)
Rabbit	New Zealand White	3F, 3M	Eye irritation	Glyphosate MEA salt	62.0 (46.6[c])	Slightly irritating	Blaszcak (1998)
Rabbit	New Zealand White	2F, 1M	Eye irritation	Glyphosate potassium salt	57.8 (47.13[c])	Slightly irritating	Bonnette (2001)
Guinea-pig	Hartley	5F, 5M	Skin sensitization (Buehler test)	Glyphosate	97.7	Not sensitizing	Auletta (1983)
Guinea-pig	Dunkin-Hartley	20F	Skin sensitization (M & K test)	Glyphosate	98.6	Not sensitizing	Cuthbert & Jackson (1989d)
Guinea-pig	Crl(HA)BR	20F	Skin sensitization (M & K test)	Glyphosate	95.6	Not sensitizing	Doyle (1996d)

F, females; IPA, isopropylamine; M, males; MEA, monoethanolamine; M & K, Magnusson & Kligman; NS, not stated;
[a] Two abraded and two intact sites
[b] Eyes were washed 20 or 30 s after treatment
[c] Purity expressed as glyphosate acid

responses scored at 24 h and 72 h. There were no signs of dermal irritation or systemic toxicity in any animal (Heenehan, 1979c). In the remaining four studies, animals with intact skin were exposed for 4 h and the responses scored at 30–60 min and 24, 48 and 72 h. In three studies, glyphosate did not produce dermal irritation (Reagan, 1988c; Cuthbert & Jackson, 1989b; Doyle, 1996b), while in one study, glyphosate produced very mild, transient dermal irritation, i.e. very slight erythema at one or both sites in five of six animals (Blaszcak, 1988c).

The potential of glyphosate acid to irritate the eye was evaluated in six studies in male and/or female New Zealand White rabbits. The studies were performed in compliance with the principles of GLP and according to the test guidelines of the US EPA or the OECD (TG 405). A volume of 0.1 ml (or 65–100 mg) of the test material was applied to one eye of each of the animals, the contralateral eye serving as the control. In one study, the test material was applied as a 25% w/v solution in distilled water (Heenehan, 1979d). In the remaining five studies, the test material was applied undiluted as wet cake (Blaszcak, 1988d) or as powder (Reagan, 1988d; Cuthbert & Jackson, 1989c; Kuhn, 1996; Johnson, 1997). The eyes were examined and scored for ocular reactions for up to 21 days after treatment.

In the first study, in the unwashed eyes there were positive scores for corneal opacity and ulceration (one out of six), for conjunctival redness (five out of six), for chemosis (one out of six) and for conjunctival necrosis (one out of six), while two out of three of the washed eyes had positive scores for corneal opacity and ulceration, conjunctival redness and chemosis. All eyes were clear of signs of irritation by day 7 (Heenehan, 1979d).

In the second study, all six animals showed moderate to severe conjunctival irritation (redness, chemosis, discharge, necrosis) and corneal ulceration, five had iritis and corneal opacities. All animals were free of ocular irritation within 7 to 14 days (Blaszcak, 1988d).

In the third study, ocular responses were corneal opacity and conjunctival irritation with blistering (six out of six), pannus of the cornea (three out of six), prominent vascularization of the conjunctiva (one out of six) and blood like discharge (one out of six). Ocular irritation persisted through study termination (day 21) in three out of five animals (Reagan, 1988d).

The fourth study was conducted in a single animal since the ocular effects (slight corneal opacity, moderate iridial responses, slight to severe conjunctival responses, slight to moderate discharge) suggested that glyphosate is severely irritant. Iridial and conjunctival responses were reversible by 96 h; however, slight cornea1 responses persisted until 96 h after instillation when the study was terminated (Cuthbert & Jackson, 1989c).

In the fifth study, the observed ocular effects (corneal opacity, conjunctival redness, chemosis) indicated severe irritation in all six unwashed eyes and moderate irritation in two out of three washed eyes. Irritation persisted through day 21 in two out of six unwashed eyes and in none of the washed eyes (Kuhn, 1996).

In the sixth study, five animals were pre-treated with a local anaesthetic before dosing since a moderate initial pain reaction was observed in the first animal dosed. Slight corneal opacity and iritis and slight to moderate conjunctival effects (redness, chemosis, discharge) were seen in all animals for up to 4 days. Irritation had completely regressed by day 7 in five animals and by day 8 in the remaining animal (Johnson, 1997).

The potential of glyphosate salts (isopropylamine, ammonium, sodium, monoethanolamine, potassium) to irritate the eye was evaluated in five studies in male and/or female New Zealand White rabbits and using test procedures as described above. The test material was applied undiluted as a liquid (Branch, 1981; Blaszcak, 1998; Bonnette, 2001) or as powder (Busch, 1987a; Busch, 1987b).

In the study with the glyphosate isopropylamine (IPA) salt, there were no signs of ocular irritation in the six animals with washed eyes as well as in the three animals with unwashed eyes up to 72 h after dosing (Branch, 1981).

In the study with the glyphosate ammonium (NH_4^+) salt, all six animals exhibited conjunctival redness, swelling, blistering and discharge within 1 h. The effects were reversible by 48 h (Busch, 1987a).

In the study with the glyphosate sodium (Na^+) salt, all six animals exhibited conjunctival redness, swelling, blistering and discharge within 1 h. The effects were reversible by 72 h (Busch, 1987b).

In the study with the glyphosate monoethanolamine (MEA) salt, one out of six animals had moderate conjunctival irritation (redness, chemosis, discharge) after 1 h, the remaining five animals had only slight conjunctival irritation. The effects were reversible by 24 h (Blaszcak, 1998).

In the study with the glyphosate potassium (K^+) salt, all three animals exhibited iritis and moderate conjunctival irritation (redness, chemosis, discharge) within 1 h. The effects were reversible by 48 h (Bonnette, 2001).

The dermal sensitization potential of glyphosate was evaluated in a Buehler test that complied with the principles of GLP. On the basis of the results of a preliminary test, groups of five male and five female Hartley Albino guinea-pigs received glyphosate (undiluted) or the positive control 2,4-dinitrochlorobenzene (DCNB) at 0.3–0.5% for both the induction phase and the challenge; additional groups of three male and three female animals were used as controls for irritation. In the induction phase, each animal received the test material three times per week for 3 weeks at a volume of 0.2 ml. Two weeks after the final induction dose, the challenge treatment was administered in the same manner. The skin reactions were scored 24 h and 48 h after removal of the patches. Beginning with the sixth induction exposure to glyphosate, mild irritation was apparent in several animals and severe irritation was seen in a few animals. No dermal responses were observed in the animals exposed to glyphosate at challenge (Auletta, 1983).

The dermal sensitization potential of glyphosate was also evaluated in a Magnusson-Kligman maximization test performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 406). On the basis of the results of a preliminary test, groups of 10 male and 10 female Dunkin-Hartley guinea pigs received glyphosate by an intradermal injection (10% w/v in distilled water) and 6 days later by topical application (25% w/v in distilled water). Slight skin irritation was observed at the treated sites. Two weeks after the topical induction, the animals were challenged with glyphosate (25% w/v in distilled water). The skin reactions were scored 24 h and 48 h after removal of the patches. None of the animals showed a positive response at challenge (Cuthbert & Jackson, 1989d).

The dermal sensitization potential of glyphosate was evaluated in another Magnusson-Kligman maximization test performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 406). On the basis of the results of a preliminary test, groups of 10 male and 10 female albino Crl(HA)BR guinea-pigs received glyphosate by an intradermal injection (0.1% w/v in deionized water) and 1 week later by topical application (75% w/v in deionized water). Slight skin irritation was observed at the treated sites. Two weeks after the topical induction, the animals were challenged with glyphosate (75% and 30% w/v in deionized water). The skin reactions were scored 24 h and 48 h after removal of the patches. Scattered mild redness was seen after challenge with the 75% w/v preparation of glyphosate in 3 out of 20 test animals and 1 out of 10 control animals; however, this response was considered to be due to skin irritation. Challenge with a 30% w/v preparation of glyphosate did not elicit a skin reaction (Doyle, 1996d).

2.2 Short-term studies of toxicity

Mice

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 408), groups of 10 male and 10 female CD-1 mice were fed diets containing glyphosate (purity, 99.5%) at a concentration that was adjusted weekly to give doses of 200, 1000 or 4500 mg/kg bw per day for 13 weeks. Animals were observed daily for symptoms of ill health and mortality. Body weights and food consumption were recorded weekly, and water consumption was monitored throughout the study. Ophthalmoscopy examinations were performed during week 12 of treatment. Blood samples were collected from the orbital sinus for haematology (seven parameters) and from the dorsal aorta at necropsy for clinical chemistry analysis (16 parameters). However, small sample volumes precluded analysis of total protein, albumin and cholesterol. At study termination, all animals were killed and necropsied, 13 organs were isolated and weighed and some 35 separate tissues were fixed for microscopy. All tissues from animals in the control group and that receiving the highest dose, in addition to the kidneys, liver and lungs of animals in the groups receiving the lowest and intermediate doses underwent a full histopathological examination.

No mortalities, clinical signs, haematological or biochemical findings and no organ weight changes were observed that could be attributed to treatment. Gross or histopathological examination did not reveal effects of glyphosate administration. Taking into account the limited range of clinical chemistry parameters evaluated, the the NOAEL was 4500 mg/kg bw per day, the highest dose tested in this study (Perry et al., 1991b).

In a study performed by the NTP, groups of 10 male and 10 female B6C3F_1 mice were fed diets containing glyphosate (purity, 99%) at a concentration of 0, 3125, 6250, 12 500, 25 000 or 50 000 ppm for approximately 13 weeks. The calculated mean time-weighted intakes were equal to 507, 1065, 2273, 4776 and 10 780 mg/kg bw per day for males and 753, 1411, 2707, 5846 and 11 977 mg/kg bw per day for females. Food and water were available ad libitum. Analyses of stability of glyphosate in the diet were performed before the start of the study. All animals were observed twice daily for mortality and morbidity. Detailed observations for clinical signs of toxicity were performed weekly. Body weights and food consumption were determined each week. At study termination, the standard haematology and clinical chemistry parameters were measured. At sacrifice, all animals were given a gross necropsy, and liver, thymus, right kidney, right testis heart and lung were

Table 12. Incidence and severity[a] of cytoplasmic alteration of the parotid salivary gland in mice fed diets containing glyphosate for 13 weeks

	Dietary concentration (ppm)					
	0	3125	6250	12 500	25 000	50 000
Males	0/10	0/10	5/10 (1.0)	9/10 (1.6)	10/10 (2.8)	10/10 (4.0)
Females	0/10	0/10	2/10 (1.0)	9/10 (1.3)	10/10 (2.4)	10/10 (3.1)

From Chan & Mahler (1992)

[a] Average severity score (given in parentheses) was based on a scale of 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

weighed. Organs and tissues were collected and preserved for histopathology. All tissues from animals in the control group and in that receiving the highest dose were examined microscopically. Salivary glands were also examined in all groups receiving lower doses.

Reduced body-weight gain was observed at 25 000 and 50 000 ppm in males and females. There were no differences in food consumption between control and treated mice. The only significant gross finding in the study was a "dark" salivary gland in a male at the highest dose; no other gross abnormalities were observed at necropsy. Histological changes were observed only in the parotid salivary gland (Table 12).

The cytoplasmic alterations consisted of a diffuse increase in the basophilia of the acinar cells. In more severely affected glands, the cells and acini also appeared to be enlarged with an appearance of reduced numbers of ducts. No histological changes were observed in the submandibular and sublingual glands.

The NOAEL was 3125 ppm (equal to 507 mg/kg bw per day) on the basis of parotid salivary gland lesions at 6250 ppm and greater, and reduced body-weight gain at 25 000 ppm and greater (Chan & Mahler, 1992).

Rats

In a range-finding study, groups of five male and five female Sprague-Dawley rats were fed diets containing glyphosate (purity, 97.7%) at a concentration of 0, 30 000, 40 000 or 50 000 ppm (equivalent to approximately 1500, 2000 and 2500 mg/kg bw per day) for 4 weeks. All animals were observed twice daily for mortality and morbidity. Detailed observations for clinical signs of toxicity were performed weekly. Body weights and food consumption were determined each week. All animals were sacrificed and given a gross necropsy at the end of the study. The liver and kidneys from each animal were preserved; for animals in the control group and at the highest dose, these organs were examined microscopically.

No animals died during the study. Slightly reduced body-weight gains were noted in both sexes at all three doses, although significant reductions consistently occurred only in males and females at the highest dose (9.6% and 9.0%, respectively, after 4 weeks). Daily food consumption was reduced for males at the intermediate and highest dose during the first week of the study. Food intake for treated females was comparable to that of controls throughout the study. The only clinical signs of toxicity were soft stools and/or diarrhoea, which occurred in both sexes at all doses with diarrhoea being the predominant sign in animals at the highest dose during the last 3 weeks of the study. Gross and microscopic pathology examinations revealed no treatment-related lesions. Because of frequent

occurrence of soft stools and/or diarrhoea at all doses, no NOAEL could be derived from this study (Reyna & Thake, 1989).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the OECD (TG 407), groups of five male and five female Sprague-Dawley rats were fed diets containing glyphosate (purity, 99.5%) at a concentration that was adjusted weekly to give doses of 0, 50, 250, 1000 or 2500 mg/kg bw per day for 4 weeks. The animals were observed daily for mortality and symptoms of ill health and once a week received a detailed clinical examination. Body weights and food consumption were calculated weekly, water consumption was monitored by visual inspection throughout the study. Blood samples were collected from the orbital sinus for haematology (seven parameters) and clinical chemistry (17 parameters) analysis. At study termination, all animals were sacrificed and necropsied. The liver, heart, kidney, spleen and adrenals were processed and examined histopathologically for all animals in the control group and at a doses of 2500 mg/kg bw per day. Examination was subsequently extended to kidneys from all females in the groups receiving the lowest and intermediate doses.

A single unscheduled death during the study occurred when a male rat from the group receiving a dose of 250 mg/kg bw per day died during blood sampling and could not be attributed to treatment with glyphosate. Soft faeces were noted in three males from the group receiving the highest dose during weeks 3–4 of the study, but were not seen in any other group. A slight but consistent body-weight reduction was observed in males and females at the highest dose of 2500 mg/kg bw per day, although statistical significance was not reached. There were no notable intergroup differences with regard to food and water consumption or haematology parameters. In males, equivocal increases in plasma alanine aminotransferase (ALT) and alkaline phosphatase (ALP) activities were seen at the three higher doses. In females, plasma ALT activity was significantly increased at the highest dose, as was total bilirubin. In addition, increased plasma concentrations of phosphate were noted in males at the two higher doses. There were neither notable intergroup differences in organ weights nor gross pathological findings. However, an increase in the incidence of very mild to slight nephrocalcinosis was observed by means of histopathology in female rats dosed at 250 mg/kg bw per day and greater (Table 13).

On the basis of the histopathological findings in kidneys and supported by changes in clinical chemistry parameters, the NOAEL was 50 mg/kg bw per day, i.e. the lowest dose tested (Atkinson et al., 1989).

Table 13. Incidence and severity of nephrocalcinosis in female rats given diets containing glyphosate for 4 weeks

	Dose (mg/kg bw per day)									
	Males					Females				
	0	50	250	1000	2500	0	50	250	1000	2500
Mineral deposits (nephrocalcinosis)	0/5	NI	NI	NI	0/5	0/5	0/5	2/5	2/5	4/5
Severity:										
Very mild/minimal	0	NI	NI	NI	0	0	0	1	1	2
Mild/slight	0	NI	NI	NI	0	0	0	1	1	2

From Atkinson et al. (1989)
NI, not investigated

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA, groups of 12 male and female Sprague-Dawley rats were fed diets containing glyphosate (purity, 95.2%) at a concentration of 0, 1000, 5000 or 20 000 ppm for 90 days. The calculated mean intakes were equal to 63, 317 and 1267 mg/kg bw per day for males and 84, 404 and 1623 mg/kg bw per day for females. Clinical signs, body weight, food consumption, haematology and clinical chemistry parameters were monitored routinely. Gross examinations were performed for all groups, and kidneys, liver, and testes were weighed. A standard range of tissues from animals in the control group and at the highest dose was examined microscopically, as well as kidneys, livers, and lungs from animals at the lowest and intermediate doses.

No treatment-related effects were observed at up to the highest dose. However, parotid salivary glands were not included in the histopathological examination. The NOAEL was 20 000 ppm (equal to 1267 and 1623 mg/kg bw per day for males and females, respectively), the highest dose tested (Stout & Johnson, 1987).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 408), groups of 10 male and 10 female Sprague-Dawley rats were fed diets containing glyphosate (purity, 98.6%) at a concentration that was adjusted weekly to give doses of 0, 30, 300 or 1000 mg/kg bw per day for 13 weeks. Animals were observed daily for symptoms of ill health and mortality. Body weights and food consumption were recorded weekly and water consumption was measured gravimetrically on a weekly basis. Ophthalmoscopy examinations were performed before the start of the study and again at week 13 of treatment. Blood samples were collected from the orbital sinus during week 13. The collected blood was analysed by haematology (eight parameters) and clinical chemistry (19 parameters). Urine samples were collected over a 4-h period of food and water deprivation, and the samples were analysed for 10 parameters. At study termination all animals were sacrificed and necropsied, 14 organs removed and weighed, and some 40 separate tissues fixed for microscopy. All tissues from animals in the control group and at the highest dose, in addition to kidneys, liver, lungs and parotid salivary glands of all the other test animals, underwent a full histopathological examination.

There were no mortalities or clinical signs throughout the 13-week study that could be attributed to the administration of glyphosate. Body-weight gain tended to be lower in males at the highest dose, but statistical significance was not attained. No such effect was seen in any other group. There were no notable intergroup differences in either sex with regard to food and water consumption or ophthalmoscopy. Haematological examinations did not reveal findings that could be related to substance administration. Females at the highest dose showed slight but statistically significant increases in concentrations of glucose (11%), total protein (9%), albumin (9%) and creatinine (8%) compared with those in the control group. Urine analysis revealed a reduction in pH in males at the highest dose. There were no intergroup differences in organ weights and no gross pathological findings that could be attributed to treatment with glyphosate.

In contrast to a 4-week study in rats conducted at the same testing facility (Atkinson et al., 1989, see above), the incidence of nephrocalcinosis in this 13-week study was evenly distributed among dose groups and sexes and did not follow a dose–response relationship, and is therefore clearly not treatment-related. Thus, the previous finding was not confirmed.

An increase in the incidence of cellular alterations (deep basophilic staining and enlargement of cytoplasm) was noted in the parotid salivary glands of both sexes in all treated groups. In addition, the severity (graded as very mild, mild, moderate, severe, and very severe) of these findings showed a dose-related increase, but reached statistical significance in males at the highest dose only (Table 14).

In conclusion, rats treated with glyphosate for 13 weeks showed dose-related histopathological changes in the parotid salivary gland. However, at the lower doses of 30 and 300 mg/kg bw per day, these changes were only minimal with respect to severity and incidence and are considered to be of equivocal toxicological significance. The NOAEL was 300 mg/kg bw per day on the basis of the more pronounced severity of cellular alterations in the parotid salivary gland at 1000 mg/kg bw per day (Perry et al., 1991a).

In a study of toxicity performed by the United States NTP, groups of 10 male and 10 female F344/N rats were fed diets containing glyphosate (purity, 99%) at a concentration of 0, 3125, 6250, 12 500, 25 000 or 50 000 ppm for approximately 13 weeks. Ten additional animals of each sex were included at each dietary concentration for evaluation of haematologic and clinical pathology parameters. The calculated mean intakes were equal to 205, 410, 811, 1678 and 3393 mg/kg bw per day for males and 213, 421, 844, 1690 and 3393 mg/kg bw per day for females. Food and water were available ad libitum. Analyses of the stability of glyphosate in the diet were performed before the start of the study. All animals were observed twice daily for mortality and morbidity. Detailed observations for clinical signs of toxicity were performed weekly. Body weights and food consumption were determined each week. At study termination, standard haematology and clinical chemistry parameters were measured. At sacrifice, all animals were given a gross necropsy, and liver, thymus, right kidney, right testis heart and lung were weighed. Organs and tissues were collected and preserved for histopathology. All tissues from animals in the control group and at the highest dose were examined microscopically. Salivary glands were also examined for animals at all lower doses.

All animals survived until the end of the study. Diarrhoea was observed in males at the highest dose and in females for the first 50 days of the study. Weight gain was reduced in males at 50 000 and 25 000 ppm, and the final mean body weight was approximately 18% and 6% less than that of controls, respectively. Females at the highest dose exhibited a 5% decrease in body-weight gain compared with the controls. Small increases in several

Table 14. Incidence and severity of cytoplasmic alteration of the parotid salivary gland in rats given diets containing glyphosate for 13 weeks

	Dose (mg/kg bw per day)							
	Males				Females			
	0	30	300	1000	0	30	300	1000
No. of animals examined	10	10	10	10	10	10	10	10
Severity:								
Very mild	3	7	6	0	2	7	7	1
Mild	0	0	3	2	0	1	2	4
Moderate	0	0	1	3	0	0	0	3
Severe	0	0	0	5*	0	0	0	1
Total incidence	3	7	10**	10**	2	8*	9**	9**

From Perry et al. (1991a)
* $p < 0.05$, ** $p < 0.01$

erythrocyte parameters were noted in males at doses of 12 500 ppm and greater. These changes were unremarkable and generally consistent with a mild dehydration. Plasma ALP and ALT activities were slightly increased in males at 6250 ppm and greater and in females at 12 500 ppm and greater. In the absence of histopathological findings in the liver, these increases are considered to be of no toxicological significance.

No treatment-related gross abnormalities or organ weight changes were observed at necropsy. Histopathological changes were observed only in the parotid and submandibular glands of male and female rats. The study authors combined the findings for these two glands (Table 15). The findings for each gland individually or for individual animals were not reported. No histological alterations were observed in the sublingual gland. The changes were described as cytoplasmic alterations and consisted of basophilic changes and hypertrophy of the acinar cells. Considering the 16-fold difference between the lowest dose of 3125 ppm and the highest dose of 50 000 ppm, the incidence–response curve appears to be relatively flat and the degree of change is slight, progressing from only minimal to moderate.

In conclusion, the administration of glyphosate to rats for 13 weeks produced dose-related histopathological changes in the parotid and submandibular salivary glands. However, at the lower doses of 3125 and 6250 ppm, these changes were only minimal with respect to severity and are considered to be of equivocal toxicological significance. The NOAEL was 6250 ppm (equal to 410 mg/kg bw per day) on the basis of the more pronounced severity of cellular alterations in the salivary glands at 12 500 ppm and greater (Chan & Mahler, 1992).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 408), groups of 12 male and 12 female Alpk:AP Wistar-derived rats were fed diets containing glyphosate (purity, 97.4%) at a concentration of 0, 1000, 5000 or 20 000 ppm for 90 days. The calculated mean intakes were equal to 81, 414 and 1693 mg/kg bw per day for males and 90, 447 and 1821 mg/kg bw per day for females. Clinical observations, body weights and food consumption were measured and all animals subjected to a full examination post mortem. Cardiac blood samples were taken and urine samples were collected for clinical pathology. Selected organs were weighed and specified tissues taken for subsequent histopathological examination. Analysis of diets showed that the achieved concentrations, homogeneity and stability were satisfactory throughout the study.

All animals survived the study in good clinical condition. A low incidence of diarrhoea and light coloured faeces were seen in both sexes at 20 000 ppm in the second week of the study. Males at the highest dose showed statistically significant reductions in

Table 15. Incidence and severity[a] of cytoplasmic alteration of the parotid and submandibular salivary glands (combined) in rats given diets containing glyphosate for 13 weeks

	Dietary concentration (ppm)					
	0	3125	6250	12 500	25 000	50 000
Males	0/10	6/10 (1.0)	10/10 (1.0)	10/10 (1.8)	10/10 (2.7)	10/10 (2.9)
Females	0/10	8/10 (1.0)	10/10 (1.0)	10/10 (2.1)	10/10 (2.4)	10/10 (3.0)

From Chan & Mahler (1992)

[a] Average severity score (in parentheses) was based on a scale of 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Table 16. Selected haematological and clinical chemistry findings at study termination (week 13) in rats given diets containing glyphosate

Parameter	Dietary concentration (ppm)							
	Males				Females			
	0	1000	5000	20 000	0	1000	5000	20 000
Platelet count × 10^9/l	708	668	638**	625	695	673	635*	625**
Prothrombin time	12.4	13.0*	13.3**	13.5**	NE	NE	NE	NE
ALT (mU/ml)	51.9	52.3	62.3*	65.2**	45.0	45.2	46.2	55.0**
ALP (mU/ml)	148	159	176*	215**	91	94	99	140**
Urea (mg/100 ml)	41.9	39.9	40.0	37.7*	40.6	40.1	42.1	35.9**
Triglycerides (mg/100 ml)	153	157	144	120**	NE	NE	NE	NE
Albumin (g/100 ml)	4.81	4.60*	4.82	4.62*	NE	NE	NE	NE
Total protein (g/100 ml)	6.53	6.22*	6.43	6.06**	NE	NE	NE	NE
Glucose (mg/100 ml)	NE	NE	NE	NE	182	183	183	208**

From Botham (1996)
ALP, alkaline phosphatase; ALT, alanine aminotransferase; NE, no treatment-related effect observed
* $p < 0.05$, ** $p < 0.01$; Student's *t*-test, two-sided

body-weight gain and food utilization efficiency when compared with controls. There was some evidence for a reduction in platelet count in males and females fed diets containing glyphosate at 5000 or 20 000 ppm. Also, a marginal dose-related increase in prothrombin time was observed in males at all doses. The differences, however, were small and considered not to be of haematological significance. Plasma ALP and ALT activities were increased in both sexes at 20 000 ppm and, to a lesser extent, in males at 5000 ppm. Further minor changes that did not always follow a clear dose–response relationship are detailed in Table 16. A few of these effects had already become apparent at week 4. In addition, plasma AST activity was increased in females at the highest dose at this early time-point, but not at study termination. There were no treatment-related effects on urine biochemistry.

The statistically significant reductions in absolute heart and liver weights in males at the highest dose were associated with lower body weights at termination. The only notable histopathological finding was a uterine leiomyosarcoma in one female at 5000 ppm. Although rare, the finding of such a tumour in an animal receiving the intermediate dose was considered to be incidental to treatment.

In the absence of other findings at 5000 ppm, the increases in plasma ALT and ALP activities in males at this dose were not considered to be of toxicological significance. The NOAEL was 5000 ppm, equal to 414 mg/kg bw per day, on the basis of reduced growth (males only) and clinical chemistry changes that may be associated with an altered liver metabolism and/or slight liver damage at 20 000 ppm (Botham, 1996).

Dogs

In a dose range-finding study performed in compliance with the principles of GLP, one male and one female beagle dogs were fed gelatin capsules containing glyphosate (purity, 99.5%) at increasing doses of 100, 300 or 1000 mg/kg bw per day, each dose being administered for 7 consecutive days. A second group of one male and one female dog received gelatin capsules containing glyphosate at a dose of 1000 mg/kg per day for 14 consecutive days. Animals were observed daily for clinical signs, body weights were recorded twice weekly, and food consumption was recorded daily. Blood, urine and faecal samples were taken before dosing and at termination. Terminal studies comprised gross examination and the weighing of heart, liver, kidneys and spleen. Specimens of these organs

plus tissue from adrenals, gonads and thymus were preserved but not evaluated microscopically.

In the first group, no treatment-related clinical signs were observed. Body weights and food consumption were considered to be satisfactory throughout the treatment period. There were no treatment-related haematological findings. A mild increase in plasma ALT activity was found in the male dog and cholesterol concentrations were slightly reduced in both animals. Studies at termination found no lesions attributable to treatment. In the second group, no treatment-related clinical signs were observed. However, loose faeces were recorded for the male dog throughout the study. Body weights and food consumption were considered to be satisfactory throughout the treatment period. There were no treatment-related haematological findings. A mild increase in plasma ALT activity was recorded in the male dog. Studies at termination found no lesions attributable to treatment (Goburdhun & Oshodi, 1989).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 409), groups of four male and four female beagle dogs were fed diets containing glyphosate (purity, 99.1%) at a concentration of 0, 2000, 10 000 or 50 000 ppm for 90 days. The calculated mean intakes were equal to 68, 323 and 1680 mg/kg bw per day for males and 68, 334 and 1750 mg/kg bw per day for females. Clinical signs including faecal consistency were recorded daily and body weights weekly. A more detailed examination including cardiac and pulmonary auscultation and indirect ophthalmoscopy was made before the start of the experiment and before termination. Food residues were recorded daily. A full range of haematology and biochemistry analyses were performed before the start of treatment and in weeks 4, 8 and 13. Urine samples were collected and analysed once before the start of the experiment and in week 13. On completion of the 90-day dosing period, all animals were killed and a full macroscopic examination carried out. Selected organs were weighed and specified tissues taken from all groups for histopathological examination. The achieved dietary concentrations of glyphosate were all within ± 9% of the target concentrations. The homogeneity of the diets was considered to be satisfactory and glyphosate was shown to be stable over 39 days.

There were no mortalities during the study. All the dogs ate all the diet presented during the dosing period. Body-weight gain of males and females at 50 000 ppm showed a slight depression throughout the study, but the differences were occasionally statistically significant only in females. There were no changes in the haematological profile attributable to treatment. In male dogs, plasma concentrations of albumin, total protein and calcium were slightly (statistically significantly) decreased at 50 000 ppm. In female dogs, plasma ALP activities were statistically significantly increased (119–125% of controls) throughout the study at 50 000 ppm. Urine analysis did not reveal indications of treatment-related findings. No adverse effects were seen at examination post mortem and no histopathological changes attributable to compound were found. Kidney weights (adjusted for body weight) of males given diets containing glyphosate at 10 000 or 50 000 ppm were statistically significantly increased (111 and 113% of controls, respectively). There was also a statistically significantly increase in liver weight (adjusted) at these doses in male dogs (111% and 113% of controls, respectively). These weight increases were not associated with any histopathological lesion.

The NOAEL was 10 000 ppm (equal to 323 mg/kg bw per day), on the basis of reduced body-weight gains and changes in clinical chemistry parameters at 50 000 ppm (Hodge, 1996).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 452), groups of four male and four female beagle dogs were fed gelatine capsules containing glyphosate (purity, 98.6–99.5%) at a dose of 0, 30, 300 or 1000 mg/kg bw per day once daily for 52 weeks. Administration of multiple capsules was necessary at the highest dose; control animals received the same number of capsules as did the group given the highest dose. The accuracy of dosing was acceptable (<5% deviation from nominal weight) and there was no indication of degradation of encapsulated glyphosate over 7 days under the storage conditions employed. The animals were observed daily for signs of ill health or reaction to the test material; observations were recorded with regard to the nature, time of onset, severity and duration. Dogs were weighed weekly, and food consumption was recorded daily. Ophthalmoscopy examinations were performed on both eyes prior to the start of dosing and again during weeks 13, 29, 39 and 51 of treatment. Laboratory investigations of haematology, clinical chemistry and urine analysis were performed on all dogs before the start of dosing and again during weeks 13, 26, 39 and 51 of treatment. Blood samples were taken from the jugular vein after the dogs had been fasted over night. Urine and faecal samples were collected over the final 17 h of a 21-h period of water deprivation while the animals were housed in metabolism cages for the conduct of kinetic investigations (determination of concentrations of glyphosate in the plasma). After completion of dosing, all the animals were sacrificed and subjected to a gross pathological examination. Seventeen organs were removed and weighed and approximately 37 tissues were processed for histopathological examination.

There were no mortalities throughout the test period. Changes in faecal consistency (soft/loose/liquid) were recorded frequently for animals in the group receiving glyphosate at a dose at 1000 mg/kg bw. This finding was observed 4–6 h after dosing and was also recorded on isolated occasions for a few animals at 300 mg/kg bw. It was considered to be related to the administration of glyphosate. There were no other clinical signs related to treatment with glyphosate. Food consumption was maximal or near maximal for all test groups. Mean body-weight gain showed a non-statistically significant reduction in males at all doses (approximately 83, 75 or 75% of that of the control group for the groups receiving the lowest, intermediate, and highest dose respectively) and in females at the highest dose (81% of that of the control group). Ophthalmoscopy and laboratory examinations revealed no treatment-related abnormalities. Plasma concentrations of glyphosate suggested that absorption was dose-related and remained constant throughout the duration of the study. Mean values detected were 0.36, 1.82 and 6.08 µg/ml for the groups receiving the lowest, intermediate and highest doses, respectively. At necropsy, no abnormal gross findings and no significant intergroup organ weight differences attributable to treatment with glyphosate were noted. In males, absolute and relative weights of the liver were slightly increased (4%, 8% and 10% above that of the control group, and 10%, 17% and 19% above that of the control group for the groups receiving the lowest, intermediate and highest doses, respectively), but the differences did not achieve statistical significance. There were no significant histopathological findings at any dose.

The faecal inconsistencies seen a few hours after dosing were most likely to be related to high local concentrations of glyphosate in the gastrointestinal tract that were attributable to the administration of the test substance in capsules. The NOAEL was 30 mg/kg bw per day on the basis of the changes in faecal consistency and the reduced body-weight gain in males at 300 mg/kg bw per day and greater (Goburdhun, 1991).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 452), groups of four male and four female beagle dogs were fed diets containing glyphosate (purity, 95.6%) at a concentration of 0, 3000, 15 000 or 30 000 ppm for 1 year. The calculated mean intakes were equal to 91, 440 and 907 mg/kg bw per day for males and 92, 448 and 926 mg/kg bw per day for females. Analysis of the diets showed that the achieved concentrations, homogeneity and stability were satisfactory throughout the study. Clinical signs were recorded daily and each dog was weighed and given a more thorough examination weekly. All dogs were given a full clinical examination (including cardiac and pulmonary auscultation and indirect ophthalmoscopy) by a veterinarian before the study, during weeks 13, 26, 39, and before termination. Food residues were recorded daily. A comprehensive range of haematology and biochemistry analyses were performed in weeks −1, 4, 13, 26 and before termination. Urine samples were collected before the start of the experiment, mid-term and during the week before termination. At the end of the scheduled period, the animals were killed and subjected to a full examination post mortem. Selected organs were weighed and specified tissues taken from all groups for histopathological examination.

There were no mortalities during the study. There was no effect on food consumption; only three dogs left small amounts of food intermittently during the study. Body weight was slightly reduced in females at 30 000 ppm, with a maximum reduction of 11% (compared with that of controls) in week 51. These dogs showed a gradual reduction in growth rate, compared with that of controls, which was consistently significant from week 23 onwards. A similar change in body-weight gain in females receiving glyphosate at the lowest dose of 3000 ppm, although occasionally reaching statistical significance, was not regarded as treatment-related since a dose–response relationship was lacking. There was no effect on body weight in males at any dose tested. There were no toxicologically significant effects on any of the haematological parameters measured. Plasma concentrations of cholesterol was slightly increased in both sexes in the treated groups at weeks 26 and 52, but there was no evidence of a dose–response relationship. Plasma concentrations of phosphorus were significantly lower in groups of treated males at week 52, which was due, in part, to slightly higher concentrations for individual control animals. The significantly reduced plasma concentration of sodium in males at the highest dose at week 52 was solely attributable to one animal. There were no treatment-related effects in any of the clinical chemical parameters measured in urine. No adverse effects of glyphosate were seen at examination post mortem and there were no treatment-related effects on organ weights. No histopathological changes attributable to administration of glyphosate were found.

The NOAEL for females was 15 000 ppm (equal to 448 mg/kg bw per day) on the basis of a reduction in body weight at 30 000 ppm. The NOAEL for males was 30 000 ppm (equal to 907 mg/kg bw per day), the highest dose tested (Brammer, 1996).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 451), groups of 50 male and 50 female CD-1 mice were fed diets containing glyphosate (purity, 98.6%) at a concentration that was adjusted weekly for the first 13 weeks and every 4 weeks thereafter to give doses of 0, 100, 300 and 1000 mg/kg bw per day for 104 weeks. Routine analysis of the diet was

performed at regular intervals throughout the study providing satisfactory results. Test animals were examined daily for mortalities and clinical signs. Once per week, animals received a detailed clinical examination with particular regard to palpable masses. Body weight and food consumption were recorded weekly until week 13 of dosing and thereafter every 4 weeks. Blood samples were collected during weeks 52, 77 and 102 and analysed for differential leukocyte counts. At study termination all surviving animals were sacrificed and necropsied, all premature decedents were also necropsied. Fifteen organs were removed and weighed and some 35 tissues were evaluated histologically for all surviving animals in the control group and at the highest dose, premature decedents were also examined. The kidneys, liver, lungs and any abnormal tissue from animals at the intermediate dose were also examined.

There were no unscheduled deaths during the course of the study that were attributable to the administration of glyphosate. Clinical signs were distributed equally throughout all the groups, and included emaciation, a hunched posture, subdued behaviour and exophthalmic eyes. There were no notable intergroup differences in the incidences of externally palpable masses. All groups receiving glyphosate showed comparable food consumption and weight gains when compared with the controls. There were no remarkable intergroup differences in differential blood counts in either sex at any of the time-points tested. The increased thymus weight in males at the intermediate and highest doses was not associated with any findings at necropsy or after histological evaluation. Owing to the slight magnitude of the increase seen, the lack of a dose–response relationship, and the lack of an effect in females, the increases were considered to be chance effects. During necropsy examinations, the incidence of lung masses was slightly higher in males at the highest dose (18/50) than in the control group (10/50); however, histopathology failed to reveal adverse lung findings. The occurrence of mineral deposits in the brain was significantly increased in males at the highest dose when compared with the control group (13/50 compared with 4/49). It should be noted that this is a common finding in mice of this age and strain. There were no other findings in the males, and no findings at all in the females that could be attributed to treatment with glyphosate.

There were no statistically significant increases in the incidence of any tumours, either benign and malignant, in either sex when compared with the control groups. However, the number of animals with multiple types of tumour was slightly higher in both sexes at the highest dose (males, 16/50; females, 11/50) than in the controls (males, 11/50; females, 6/50). This led to a slight increase in the total number of tumours in the at the highest dose for both sexes (males, 60; females, 43) compared with the controls (males, 49; females, 36). Haemangiosarcoma was evident in 4/50 males at the highest dose, in 2/50 females at the lowest dose, and in 1/50 females at the highest dose, but in none of the 50 animals of the control group. Histiocytic sarcoma in the lymphoreticular/haemopoietic tissue was evident in 2/50 males at the lowest and highest doses, and in 3/50 females at the lowest and intermediate doses and 1/50 females at the highest dose when compared with the respective controls (0/50). Owing to the lack of a dose–response relationship, the lack of statistical significance and the fact that the incidences recorded in this study fell within the historical ranges for controls, these changes are not considered to be caused by administration of glyphosate.

In conclusion, administration of glyphosate to CD-1 mice for 104 weeks produced no signs of carcinogenic potential at any dose. The NOAEL was 1000 mg/kg bw per day, the highest dose tested (Atkinson et al., 1993a).

Rats

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 452), groups of 24 male and 24 female Alpk:AP_fSD (Wistar-derived) rats were given diets containing glyphosate (purity, 95.6%) at a concentration of 0, 2000, 8000 or 20 000 ppm for 1 year. Analysis of diets showed that the achieved concentrations, homogeneity and stability were satisfactory throughout the study. The calculated mean intakes were equal to 141, 560 and 1409 mg/kg bw per day for males and 167, 671 and 1664 mg/kg bw per day for females. The animals were monitored daily for mortality and clinical observations. Body weights and food consumption were measured and, at the end of the scheduled treatment period, the rats were killed and subjected to a full examination post mortem. Blood and urine samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

There were no unscheduled deaths during the course of the study that could be attributed to the administration of glyphosate. Apart from a small increase in the number of male and female animals in the group receiving gyphostae at 20 000 ppm that showed wet or dry urinary staining, there were no other treatment-related clinical observations and no treatment-related ophthalmological findings. At the two higher doses, body weights were lower than those of the concurrent controls, with the difference reaching statistical significance at 20 000 ppm in both sexes and at 8000 ppm only in females towards study termination. There was no effect on body weight in animals at 2000 ppm. Food consumption was lower and food utilization was slightly less efficient at 20 000 ppm, the reductions being most marked at the start of the study. There was a trend for reduced food intake for females at 8000 ppm, which correlates with the reduction in body-weight gain at this dose in the latter stages of the study.

Deviations in some clinical chemistry parameters, such as reductions in plasma concentration of cholesterol and triglycerides or a dose-related increase in plasma ALP activity throughout the study as well as occasional increases in the activities of plasma AST, ALT and creatine kinase, were mostly confined to groups receiving the high and intermediate doses and were probably treatment-related (Table 17). In the absence of any histopathological findings, these changes are considered to be of marginal toxicological relevance. There was no evidence of any effect of glyphosate on urine parameters.

At necropsy, there were no gross pathological findings that could be attributed to treatment and no consistent organ weight changes. Histopathology revealed an increased incidence and severity of focal basophilia of the acinar cells of the parotid salivary gland in both sexes at 20 000 ppm (Table 18). At 8000 ppm, this finding was of minimal severity and its incidence was only slightly above that in the control animals. No other microscopic findings could be ascribed to administration of glyphosate.

Similar numbers and types of neoplasms were diagnosed in the control group and in the group receiving glyphosate at 20 000 ppm, but the duration of the study was not sufficiently long to enable final conclusions to be made with regard to carcinogenicity. The NOAEL was 2000 ppm, equal to 141 mg/kg bw per day, on the basis of a reduction in body weight and clinical chemistry findings at dietary concentrations of 8000 ppm and greater (Milburn, 1996).

Table 17. Selected clinical chemistry findings in rats given diets containing glyphosate for 1 year

Parameter	Dietary concentration (ppm)							
	Males				Females			
	0	2000	8000	20 000	0	2000	8000	20 000
Cholesterol:								
Week 14	2.46	2.53	2.31	2.28*	2.13	2.28	2.26	2.21
Week 27	3.09	3.05	2.75*	2.70**	2.62	2.67	2.76	2.78
Triglycerides:								
Week 14	1.56	1.63	1.28**	1.28**	0.94	0.92	0.89	0.95
Week 27	1.51	1.43	1.15**	0.97**	1.07	1.10	1.13	1.10
ALP:								
Week 14	248	281	342**	429**	161	201*	227**	292**
Week 27	221	250	306**	412**	135	171	200**	254**
Week 53	232	258	291**	379**	87	100	114	160**
ALT:								
Week 14	84	93	111**	110**	66	79	88**	91**
Creatine kinase:								
Week 14	118	124	127	144**	97	108	107	124**

From Milburn (1996)
ALP, alkaline phosphatase; ALT, alanine aminotransferase
*$p < 0.05$, **$p < 0.01$; Student's *t*-test, two-sided

Table 18. Incidence of focal basophilia of parotid acinar cells in rats given diets containing glyphosate for 1 year

	Dietary concentration (ppm)							
	Males				Females			
	0	2000	8000	20 000	0	2000	8000	20 000
Severity:								
Minimal	2	0	3	10	2	0	6	8
Slight	0	0	0	3	0	0	0	5
Moderate	0	0	0	0	0	0	0	2

From Milburn (1996)

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA, groups of 60 male and 60 female Charles River CD®(SD)BR rats were fed diets containing glyphosate (purity, 96.5%) at a concentration of 0, 2000, 8000 or 20 000 ppm for 2 years. In principle, the study was also compliant with OECD TG 453, although satellite groups comprised only 10 animals of each sex and group and survival had fallen below 50% (varying between 29% and 44%) in all test groups at scheduled termination. Regular dietary analyses gave satisfactory actual concentration, homogeneity and stability. The calculated mean intakes were equal to 89, 362 and 940 mg/kg bw per day in males and 113, 457 and 1183 mg/kg bw per day in females. All animals were observed twice daily for mortality and moribundity and detailed observations for clinical signs of toxicity were performed weekly. Body weights and food consumption were determined each week for the first 13 weeks and then every fourth week thereafter. Ophthalmic examinations were performed before the test and just before terminal sacrifice. Comprehensive determinations of haematological, blood biochemistry and urine analysis parameters were conducted on 10 animals of each sex per dose each at 6, 12 (interim sacrifice), 18, and 24 (study termination) months. Ten animals of each sex per dose were sacrificed at month 12. All animals were given a complete gross necropsy. Brain, kidneys, liver and testes with epididymides were weighed. Approximately 40 tissues were preserved and examined microscopically.

There were no statistically significant differences in mortality during the study. No evidence of treatment-related clinical signs was recorded except the ophthalmological findings mentioned below. Statistically significant reductions in body weight were noted in females at the highest dose from week 7 to approximately month 20. During this time, absolute body weights gradually decreased to 14% below the control value owing to a reduction in body-weight gain by up to 23%. In contrast, body-weight gain in all treated male groups was comparable to that of controls. Food consumption was not adversely affected by treatment in any sex despite an increase in males at the highest dose.

The ophthalmic examination before study termination revealed a statistically significant difference ($p < 0.05$) between the incidences of cataractous lens changes in males in the control group and in the group receiving the highest dose (none out of 15 compared with five out of 20). The occurrence of cataractous lens changes in males at the lowest and intermediate doses, as well as in all treated groups of females, were comparable to that of their respective controls. The observed incidence for this finding of 25% for male CD rats at the highest dose was within the range (0–33%) observed in previously conducted studies at this laboratory, but a treatment-related impact could not be excluded. An independent pathologist's examination confirmed a statistically significant increase ($p < 0.05$) in the incidence of cataractous lens changes in males at the highest dose (one out of 14 compared with eight out of 19) and concluded that there appeared to be a treatment-related occurrence of lens changes affecting males at the highest dose. Histological examination of the eyes at study termination revealed the incidences of cataract and/or lens fibre degeneration (Table 19). The results of histopathology also suggested that there was an increase in cataractous lesions in male rats at 20 000 ppm, although the difference in incidences in the control group and at the lowest and intermediate doses was less pronounced than suggested by ophthalmoscopy.

This outcome was essentially confirmed by re-evaluation by an independent laboratory. It was concluded that there was a slight, statistically significant (as indicated in the Cochrane-Armitage linear trend test) increased incidence of basophilic degeneration of the posterior subcapsular lens fibres in males at the highest dose, but not in those at the intermediate or lowest dose, nor in any treated group of females.

There were various changes in haematology and serum chemistry parameters, but these changes were not consistently noted at more than one time-point, were within ranges for historical controls, were small in magnitude, and/or did not occur in a dose-related manner. Therefore, they were considered to be either unrelated to treatment or toxicologically not significant. However, the statistically significant increase in alkaline phosphatase activity in females at the highest dose at study termination is in line with observations made in other long-term studies in rats, although it was partly attributable to one animal with an

Table 19. Incidences of cataract and lens fibre degeneration determined by histological examination in male rats given diets containing glyphosate for 1 year

	Dietary concentration (ppm)			
	0	2000	8000	20 000
Terminal sacrifice	2/14	3/19	3/17	5/17
All animals	4/60	6/60	5/60	8/60

From Stout & Ruecker (1990)

outstandingly high value. Statistically significant reductions in urine pH were noted in males at the highest dose at months 6, 18, and 24, reflecting the renal excretion of glyphosate, which is an acid.

Statistically significant increases in liver weight were noted in males at the highest dose. There were no other statistically significant changes in organ weights that occcurred in a dose-related manner. Gross abnormalities observed at necropsy were not considered to be related to administration of glyphosate.

Regarding neoplastic lesions, the only statistically significant difference between control and treated animals was an increase in the incidence of pancreatic islet cell adenomas in males at the lowest dose. The incidences of this lesion were 1 out of 58 (2%), 8 out of 57 (14%), 5 out of 60 (8%), and 7 out of 59 (12%) in males in the control group and at the lowest, intermediate and highest dose, respectively. The historical-control range for this tumour at the testing laboratory was 1.8–8.5%, but a partial review of studies reported recently in the literature revealed a prevalence of 0–17% in control males with several values being ≥ 8%. More importantly, the incidences of islet cell adenomas clearly did not follow a dose-related trend in the treated groups of males, as indicated by the lack of statistical significance in the Peto trend test. It should be noted that there was also considerable inter-group variability in the numbers of females with this tumour (5 out of 60, 1 out of 60, 4 out of 60 and 0 out of 59 in the control group and at the lowest, intermediate and highest doses, respectively). There was no evidence of dose-related pancreatic damage or pre-neoplastic lesions. The only pancreatic islet cell carcinoma found in this study occurred in a male in the control group, thus indicating a lack of treatment-induced neoplastic progression. Taken together, the data support the conclusion that the occurrence of pancreatic islet cell adenomas in male rats was spontaneous in origin and unrelated to administration of glyphosate.

With regard to non-neoplastic changes (apart from the findings in the eye, described above), histopathological examination revealed an increase in the number of animals displaying inflammation of the stomach squamous mucosa at 8000 and 20 000 ppm, achieving statistical significance for females only at the intermediate dose. The incidences of this lesion in all groups of animals are shown in Table 20.

Although the incidence of this lesion in females at the intermediate dose (15%) was slightly outside the range for historical controls (0–13.3%) for the laboratory, there was no

Table 20. Incidence of inflammation and hyperplasia of the stomach squamous mucosa in rats given diets containing glyphosate for 24 months

	Dietary concentration (ppm)			
	0	2000	8000	20 000
Males				
Inflammation	2/58	3/58	5/59	7/59
Hyperplasia	3/58	3/58	4/59	7/59
Females				
Inflammation	0/59	3/60	9/60**	6/59
Hyperplasia	2/59	3/60	7/60	6/59

From Stout & Ruecker (1990)
** $p \leq 0.01$; Fisher's exact test with Bonferroni inequality

dose-related trend across all groups of treated females and there was no significant difference in any group of males. Therefore, it is equivocal whether this finding was treatment-related. However, a weak irritation potential of the test material may be assumed at high doses. In contrast to other long-term studies, no histological changes in salivary glands were reported. However, it must be mentioned that in this study only the mandibular (submaxillary) salivary glands were evaluated microscopically, and not the parotid glands.

In conclusion, administration of glyphosate to Sprague-Dawley rats for 24 months produced no signs of carcinogenic potential. The NOAEL was 8000 ppm, equal to 362 mg/kg bw per day, on the basis of a reduction in body weight in females and cataractous lens changes in males at 20 000 ppm (Stout & Ruecker, 1990).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA, groups of 85 male and 85 female Sprague-Dawley rats were fed diets containing glyphosate (purity, 98.7–98.9%) at a concentration that was adjusted weekly for the first 12 weeks and every 2 months thereafter to give doses of 0, 100, 300 and 1000 mg/kg bw per day for 104 weeks. The doses were selected on the basis of results from a 13-week dietary study of toxicity. Routine analysis of the diet was performed at regular intervals throughout the study, giving acceptable results. Fifty rats of each sex per dose were allocated to the 104-week study of oncogenicity, and 35 rats of each sex per dose were allocated for long-term testing for toxicity. Fifteen rats of each sex per dose from every group testing for toxicity in the long term were killed after 52 weeks, all remaining rats were dosed until scheduled termination after 104 weeks. Test animals were examined daily for mortalities and clinical signs. Once per week, animals received a detailed clinical examination, with particular regard to palpable masses. Body weight and food consumption were recorded weekly until week 13 of dosing and thereafter every 4 weeks. Ophthalmoscopy examinations were performed on 20 males and 20 females from the control groups and the group receiving the highest dose before initiation of dosing and again during weeks 24, 50 and 102. Blood samples were collected from the orbital sinus of 10 males and 10 females from each group after approximately 14, 25, 51, 78 and 102 weeks. Blood samples were analysed for eight haematology parameters and nineteen clinical chemistry parameters. At study termination, all surviving animals (and the premature decedents, if possible) were sacrificed and necropsied. Fifteen organs were removed and weighed and some 35 tissues were evaluated histologically from all surviving animals in the control group and at the highest dose, premature decedents being also examined in this way. The kidneys, liver, lungs, sublingual, submaxillary and parotid salivary glands and any abnormal tissue from the groups receiving the intermediate dose were also examined.

Survival was not affected by treatment and there were no clinical signs of toxicity that were thought to be related to administration of glyphosate. Ophthalmoscopy did not reveal any indications of adverse effects. Body-weight gain was reduced in males and females at the highest dose. At the lower doses, no consistent and clearly dose-related body-weight change was to be seen. Food consumption and water intake were not affected.

Haematological changes were not considered to be treatment-related, although erythrocyte volume fraction and haemoglobin were occasionally increased in males and females at the highest dose. However, a similar increase was also observed at other doses, in particular in males receiving a dose of 100 mg/kg bw per day, and a clear dose–response relationship was lacking. In addition, the differences observed were rather small and no consistent trend became obvious throughout the study. In contrast, clinical chemistry

investigations and urine analysis elucidated some changes that could be attributed to administration of the compound. An increase in plasma ALP activity became most apparent in males and females at the highest dose, but was also noted and occasionally reached statistical significance at the intermediate doses of 300 and 100 mg/kg bw per day (Table 21). All other changes in clinical chemistry were not considered to be unequivocally treatment-related. Urine pH was consistently decreased in males at the highest dose and tended to be lower from a dose of 100 mg/kg bw per day onwards. However, a similar effect was not observed in females.

At interim sacrifice after 52 weeks, absolute weight of the liver was reduced at doses of 1000, 300 and 100 mg/kg bw per day. For males, however, this finding was not confirmed by the sensitive means of covariance analysis, i.e. with correction for final body weight. At terminal sacrifice, no statistically significant decrease in liver weight was noted. In contrast, mean weight of the kidney was reduced in groups of males at 100 and 1000 mg/kg bw after 104 weeks, but a clear dose–response relationship was lacking. A probably treatment-related impact on weight of the salivary gland was noted in both sexes at interim kill. At study termination, weight of the submaxillary (mandibular)/sublingual glands in both sexes and of the parotid salivary gland (females only) still tended to be higher at the two higher doses. However, when compared with these values in the controls, the difference was rather small, statistical significance was not achieved and there was no clear dose–response relationship.

Gross necropsy did not reveal indications of treatment-related non-neoplastic changes. The only remarkable histopathological finding attributed to administration of glyphosate was a dose-related increase in the number of animals exhibiting cellular alteration of the parotid and mandibular (submaxillary) salivary glands at the highest dose and at both intermediate doses. The changes were seen after 52 weeks. This alteration was described as the occurrence of hypertrophic and weakly (mandibular gland) or more deeply (parotid gland) basophilic acinar cells without any evidence of degeneration or other toxic damage. The severity of alteration was graded by the study pathologist on a scale ranging from "slight" to "very severe" (slight/very mild, mild, moderate, severe, very severe). The changes graded as "moderate" or "severe" were seen more frequently at 300 and 1000 mg/kg bw per day (Table 22). The sublingual salivary gland was not affected.

Neoplasia was present in all groups, but there was no relationship with dose in the incidence of any individual tumour or in the total incidence of animals with tumours.

Table 21. Plasma alkaline phosphatase (ALP) activity (IU/l) in rats given diets containing glyphosate for 104 weeks

Time-point	Dose (mg/kg bw per day)									
	Males					Females				
	0	10	100	300	1000	0	10	100	300	1000
Week 14	287	229	320	334	461***	182	158	213	223	244*
Week 25	251	272	267	306	367***	148	152	201*	227**	225**
Week 51	308	293	310	353	403	144	143	190*	195*	221**
Week 78	258	286	284	351*	414***	124	139	172	207**	186*
Week 102	212	265	287*	267	365***	190	161	193	228	286*

From Atkinson et al. (1993b)
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 22. Incidence and severity of cellular alteration of salivary glands in rats given diets containing glyphosate for 104 weeks

	Dose (mg/kg bw per day)									
	Males					Females				
	0	10	100	300	1000	0	10	100	300	1000
No. of animals examined	50	46	49	50	49	50	50	50	50	48
Parotid										
Severity:										
Slight	4	4	8	3	4	1	2	2	2	5
Mild	3	5	9	21***	14**	0	5	9**	9**	13***
Moderate	0	0	4	17***	18***	1	1	1	9*	18***
Severe	0	0	0	0	0	0	0	0	1	2
Total incidence	7	9	21**	41***	36***	2	8	12**	21***	18***
Mandibular										
Severity:										
Slight	7	5	10	14	9	2	0	3	1	6
Mild	0	0	12***	28***	22***	9	8	9	15	19**
Moderate	0	0	0	0	0	0	0	0	2	1
Total incidence	7	5	22***	42***	31***	11	8	12	18	26**

From Atkinson et al. (1993b)
** $p < 0.01$, *** $p < 0.001$

In conclusion, administration of glyphosate to rats for 104 weeks produced no evidence of a carcinogenic response. The liver and the salivary glands were identified as the main target organs of glyphosate-related toxicity in the long term. At 100 mg/kg bw per day, the changes in salivary glands were only minimal with respect to severity and were not considered to be of toxicological significance. Thus, the NOAEL was 100 mg/kg bw per day on the basis of the more pronounced cellular alteration of salivary glands at 300 mg/kg bw per day and greater (Atkinson et al., 1993b).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 453), groups of 52 male and 52 female Alpk:AP$_f$SD (Wistar-derived) rats were fed diets containing glyphosate (purity, 97.6%) at a concentration of 0, 2000, 6000 or 20 000 ppm for 2 years. A further 12 males and 12 females were added to each group and were designated for interim kill after 1 year. Achieved concentration was assessed regularly and the stability and homogeneity of glyphosate in the diet were determined and found to be satisfactory. The calculated mean intakes were equal to 121, 361 and 1214 mg/kg bw per day in males and 145, 437 and 1498 mg/kg bw per day in females. Clinical observations (including ophthalmoscopy), body weights, food consumption, haematology and clinical biochemistry (blood and urine), were measured throughout the study. In addition, a functional observational battery (FOB), including motor activity, was conducted in week 52 in animals allocated to the long-term assessment of toxicity part of the study. At the end of the scheduled period, the animals were killed and subjected to a full examination post mortem. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues taken for subsequent histopathological examination.

Survival in males in the control group and in groups receiving the lowest and intermediate doses approached 25% by week 104 of the study, although survival at the highest dose was significantly better. Survival in females was similar across all groups and better than in males in the group receiving the lower dose. There was a treatment-related increase in the incidence of red-brown staining of tray papers (particularly in males), and isolated

observations of red-brown coloured urine noted in three males and one female at 20 000 ppm. No other treatment-related clinical signs of toxicity (including ophthalmoscopic findings and FOB) occurred. The body weights of males and females at 20 000 ppm were statistically significantly lower than those of the controls throughout the study; however, the difference was rather small with a maximum reduction compared with control values of approximately 5% for males and 8% for females. There were no dose-related and/or statistically significant effects on body weight in males or females at 2000 or 6000 ppm. The decrease in body weight at the highest dose was paralleled by lower food consumption throughout the first year of the study in males and females and an impaired food utilization in these groups during weeks 1–4.

Minor variations from mean values for the controls were obtained for most haematological parameters, but showed no consistency and were confined to intermediate time-points and/or doses and these changes were thus considered not to be treatment-related. In contrast, some clinical chemistry findings were assumed to be caused by administration of glyphosate, at least at the highest and intermediate doses. There was a clear dose-related increase in plasma ALP activity in both sexes throughout the study reaching statistical significance at the two higher doses (Table 23). In the groups at 2000 ppm, the mean values also tended to be higher; however, the increase was marginal and only occasionally achieved statistical significance (in males at week 79 and in females at week 53). In addition, there was evidence of increases in plasma ALT and AST activities, and in total bilirubin concentration at one or more time-points. These findings were confined to the groups receiving the intermediate and highest doses and frequently occurred in one sex only. In males at the highest dose, plasma concentrations of triglycerides and cholesterol were consistently decreased throughout the study. Plasma concentrations of creatinine were lower in all treated female groups at week 27 and in females receiving 6000 and 20 000 ppm at week 14, but in the absence of any effects later in the study, this was considered as having occurred by chance rather than suggesting an adverse effect. In males at the highest dose of 20 000 ppm,

Table 23. Plasma alkaline phosphatase (ALP) and alanine aminotransferase (ALT) activities in rats given diets containing glyphosate for 2 years

	Dietary concentration (ppm)							
	Males				Females			
	0	2000	6000	20 000	0	2000	6000	20 000
ALP (IU/l):								
Week 14	234	246	284**	387**	156	177	245**	266**
Week 27	196	219	239**	327**	121	136	166**	203**
Interim kill	230	244	269	306**	82	102	123*	144**
Week 53	231	249	277**	357**	92	117*	152**	172**
Week 79	208	254*	244	353**	114	131	181**	178**
Week 105	184	205	218	280	144	129	158	173
ALT (IU/l):								
Week 14	94.9	103.5	121.8**	143.4**	81.9	95.2	103.9*	94.9
Week 27	91.8	95.9	116.8	125.9*	99.5	113.8	132.7*	101.8
Interim kill	77.6	84.0	97.7	123.3**	83.4	82.8	113.2*	95.9
Week 53	84.2	99.8	103.5	133.8**	90.1	108.2	121.5*	114.0
Week 79	69.2	81.2	102.4**	105.9**	90.0	97.2	110.6	116.0*
Week 105	64.1	58.6	63.9	82.7	83.5	78.6	78.9	108.2**

From Brammer (2001)
ALP, alkaline phosphatase; ALT, alanine aminotransferase
* $p < 0.05$, ** $p < 0.01$; Student's *t*-test, two-sided

urinary pH was lower than in controls throughout the study, and the incidence and number of erythrocytes in the urine was increased in males and, to a lesser extent, in females.

No increase in tumour incidence was observed. A number of probably treatment-related macroscopic findings were seen in males at 6000 ppm and/or 20 000 ppm, consisting of a minor increase in the incidence of enlarged kidneys, single masses in the liver, firmness of the prostate and a reduction in the incidence of reduced testes. However, there were no consistent, dose-related and/or statistical significant organ-weight changes that could be considered to indicate an adverse effect of glyphosate.

In contrast to the previous 1-year feeding study in rats (Milburn, 1996, see above) that was performed in the same laboratory and on the same rat strain, microscopic changes were seen in the liver and kidneys, but not the salivary glands of rats at 20 000 ppm (Table 24). Changes in the liver comprised a weak and rather equivocal increase in the incidence of hepatitis (evidence obtained in male only) and proliferative cholangitis, but the severity of the latter finding was not altered. There were a number of changes in the kidneys of both sexes, notably renal papillary necrosis, with or without papillary mineralization, and transitional cell hyperplasia. The incidence was greater in males than females. These renal findings were considered to be related to treatment but are consistent with the feeding of high doses of an acidic material, which may also have caused the microscopically observed prostatitis and periodontal inflammation observed. A decrease in the incidence of tubular degeneration of the testis in males at 20 000 ppm was considered to be without adverse consequence.

In conclusion, dietary administration of glyphosate at up to the highest dietary concentration of 20 000 ppm for up to 2 years produced little evidence of toxicity in the long term, with the kidney, the prostate and possibly the liver being the target organs. A number of findings (e.g. renal papillary necrosis, prostatitis, periodontal inflammation and urinary acidosis) might be attributed to the acidity of the test substance. No indications of neurotoxicity were obtained. The improved survival in males at the highest dose was likely to be associated with lower food consumption, lower body weights and a decreased severity of renal glomerular nephropathy. In the absence of treatment-related histopathological findings at 2000 and 6000 ppm, the marginal changes in some clinical chemistry parameters at these doses were considered to be of no toxicological significance.

Table 24. Selected microscopic findings in rats given diets containing glyphosate for 2 years

Finding	Dietary concentration (ppm)							
	Males (n = 64)				Females (n = 64)			
	0	2000	6000	20 000	0	2000	6000	20 000
Liver:								
Proliferative cholangitis	56	57	55	64	55	58	59	61
Hepatitis	8	6	9	13	6	7	4	6
Kidney:								
Papillary necrosis	0	1	0	14	0	1	2	5
Transitional cell hyperplasia	2	3	0	5	3	1	0	1
Prostate: prostatitis	13	22	23	37	—	—	—	—
Testis: unilateral tubular degeneration	18	13	18	5	—	—	—	—
Periodontal inflammation	25	27	23	42	18	24	32	28

From Brammer (2001)

Administration of glyphosate for 2 years produced no evidence of a carcinogenic response to treatment in rats. The NOAEL was 6000 ppm (equal to 361 mg/kg bw per day), on the basis of a reduction in body weight and food consumption, and indications for kidney, prostate and liver toxicity at 20 000 ppm (Brammer, 2001).

2.4 Genotoxicity

Glyphosate has been extensively tested for genotoxicity in a wide range of assays both in vitro and in vivo, including end-points for gene mutation, chromosomal damage and DNA damage and repair. Numerous studies of genotoxicity have been reported, including many publications with limited experimental details, partly contradictory results and the technical specification of test material often being unknown. Data of very different quality are available for glyphosate as the acid but also for the salts (e.g. the isopropylamine salt) and also for different plant protection products (formulations). However, in this review the focus is on mutagenic properties of the active substance. The results of the available regulatory studies with the active ingredient using test material corresponding to the Food and Agriculture Organization of the United Nations (FAO) specification were uniformly negative. The few published data (mostly obtained in vitro) suggesting positive results in validated and widely accepted test systems are contradicted by the vast majority of studies with clearly negative outcomes. More important, the studies in standard test systems in vivo clearly proved the lack of mutagenic effects. Thus, it may be concluded that glyphosate active ingredient is devoid of a relevant genotoxic potential. The experimental data on which this assessment relies are summarized in Tables 25 and 26.

In vitro, glyphosate gave negative results in a number of assays for gene mutation in bacteria across a wide range of concentrations in the tester strains of *S. typhimurium* and *E. coli*. The compound also gave negative results in assays for gene mutation in mammalian cell systems at both the *Tk* and *Hgprt* loci. Glyphosate was non-clastogenic in standard guideline assays for chromosome aberrations and damage. These negative results for gene mutation and clastogenicity have been generated in both the absence and presence of metabolic activation (S9).

In contrast, investigations designed to examine the effects of long-term exposure to glyphosate on cells in culture have reported positive findings for the induction of chromosomal aberrations and sister chromatid exchange (SCE), but these have been ascribed as a likely consequence of the perturbation of the homeostasis of the cells. Some reports of increases in SCE frequencies have involved very small numerical increases, albeit attaining statistical significance. This is frequently seen with SCE as an end-point. Overall, the available data show that glyphosate was non-clastogenic when evaluated in appropriate assays for chromosomal damage. Glyphosate did not induce DNA repair as measured in both bacterial (Rec assay) and mammalian systems (UDS assay). The in-vitro studies submitted for this review are summarized in Table 25.

Glyphosate has been extensively investigated for clastogenic activity in vivo, in mice mainly by assay for micronucleus formation in the bone marrow. These data show that glyphosate is non-clastogenic after both single and repeated administration. An isolated weakly positive finding after intraperitoneal injection is contradicted by other results including all studies using the more relevant oral route of administration. Likewise, glyphosate proved non-clastogenic in the assay for cytogenetic damage (metaphase) in bone marrow in rats. Glyphosate can also be considered to be non-mutagenic to germ cells, giving a

Table 25. Results of studies of genotoxicity with glyphosate in vitro

End-point	Test object	Concentration	Purity (%)	Results	Reference
Reverse mutation[a]	*S. typhimurium* TA1535, TA1537, TA98 and TA100 and *E. coli* WP2P and WP2P*uvrA* ± S9	100, 200, 500, 1000, 2500, 5000 µg/plate in DMSO	95.6	Negative	Callander (1996)
Reverse mutation[a]	*S. typhimurium* TA1535, TA1537, TA98, TA100	310–5000 µg/plate (+S9); 160–2500 µg/plate (−S9)	98.6	Negative	Jensen (1991a)
Reverse mutation[b]	*S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538, and *E. coli* WP2 ± S9	10–5000 µg/plate	98.4	Negative	Shirasu et al. (1978) (published by Li & Long, 1988)
Reverse mutation[c]	*S. typhimurium* TA98, TA100, TA1535, TA1537 ±S9	≤1000 µg/plate	98.4	Negative	Kier (1978)
Reverse mutation	*S. typhimurium* TA97a, TA98, TA100 and TA102 ±S9	25–2000 µg in aqueous solution	Not specified ("technical concentrate")	Negative	Chruscielska et al. (2000)
Reverse mutation	*S. typhimurium* TA97, TA98, TA100 and TA1535 ±S9	0–10 000 µg/plate	99	Negative	Chan & Mahler (1992)
Point mutation[a]	Mouse lymphoma L5178Y $Tk^{+/-}$ cells ±S9	444, 667, 1000 µg/ml	95.6	Negative	Clay (1996)
Point mutation[d]	Mouse lymphoma L5178Y $Tk^{+/-}$ cells ±S9	0.52–4.2 mg/ml (+S9); 0.61–5.0 mg/ml (−S9)	98.6	Negative	Jensen (1991b)
Point mutation[c]	Chinese hamster ovary cells, HGPRT locus, ±S9	5–25 mg/ml (+S9); 5–22.5 mg/ml (−S9)	98.7	Negative	Li (1983a) (published by Li & Long, 1988)
Cytogenetic damage	Human lymphocyte cultures from male and female donors ±S9; two harvest times	100, 750, 1250 µg/ml	95.6	Negative	Fox (1998)
Cytogenetic damage	Human lymphocyte cultures; exposure time 24 & 48 h (−S9) or 3 h (+S9); harvest after 24 or 48 h	237–562 µg/ml (+S9); 33–333 µg/ml (−S9)	96	Negative	Van de Waart (1995)
Sister chromatid exchange	Human lymphocytes	0–6 mg/ml	99.9	Positive at ≥1 mg/ml	Bolognesi et al. (1997) Insufficient data for adequate assessment.
Chromosomal aberration and sister chromatid exchange	Bovine lymphocyte cultures, 72-h treatment	17, 85, 170 µmol/l	≥98	Positive at all three concentrations	Lioi et al. (1998a)[f]
Chromosomal aberration and sister chromatid exchange	Human peripheral lymphocytes, 72-h treatment	5.0, 8.5, 17.0, 51.0 µmol/l	≥98	Positive at 8.5, 17.0 and 51.0 µmol/l	Lioi et al. (1998b)
DNA damage (Rec assay)[g]	*B. subtilis* strains H17 (rec+) and M45 (rec-)	20–2000 µg/disc	98.4	Negative	Shirasu et al. (1978) (published by Li & Long, 1988)
Unscheduled DNA synthesis[c]	Hepatocytes from F344 rats	≤125 µg/ml	98.7	Negative	Williams (1983) (published by Li & Long, 1988)

[a] A positive control was employed; GLP and QA statements included. Complied with current regulatory guidelines
[b] This study was performed before the publication of current guidelines and before GLP, however the protocol generally adhered to these guidelines and is considered acceptable
[c] The study was performed before GLP but is considered acceptable
[d] Study complied with GLP and is considered acceptable, QA statements are included
[e] The study was conducted to GLP (self-certification of the laboratory). The study is considered acceptable
[f] Non-standard test system; effects ascribed to a likely alteration in the oxidative state of the treated cells after long exposure
[g] The study was performed before GLP and was not conducted in the presence of metabolic activation

negative result in an assay for dominant lethal mutation in mice. Tests for DNA damage using alkaline elution in the liver and kidney of mice are of limited value on account of the use of the intraperitoneal route of administration and the lack of appropriate data on toxicity. Similar concerns apply to studies on DNA binding from the same laboratory, although no adducts were identified after administration of glyphosate isopropylamine salt. The results of the studies of genotoxicity provided are summarized in Table 26.

Table 26. Results of studies of genotoxicity with glyphosate in vivo

End-point	Test object	Concentration	Purity (%)	Result	Reference
Micronucleus formation[a]	Charles River CD-1 mice (males and females), bone marrow; sampling at 24 h and 48 h after dosing	5000 mg/kg bw (single oral dose)	95.6	Negative	Fox & Mackay (1996)
Micronucleus formation[b]	NMRI mice (males and females, bone dose) marrow; sampling after 24 h, 48 h and 72 h	0–5000 mg/kg bw (single oral)	98.6	Negative	Jensen (1991c)
Micronucleus formation	B6C3F1 mice (males/ females), peripheral (normochromatic, blood erythrocytes	0–50 000 ppm (examination after dietary administration for) 13 weeks)	Not stated	Negative	Chan & Mahler (1992)
Micronucleus formation	Swiss CD-1 mice (males only), bone marrow. Sampling 6 h and 24 h after final dose.	300 mg/kg bw (2 × 150 mg/kg bw); intraperitoneal administration	99.9	Weakly positive after 24 h	Bolognesi et al. (1997)
Micronucleus formation	Mice (strain not specified, males only), bone marrow; Sampling time 24, 48 and 72 h after dosing	300 mg/kg bw (single intraperitoneal injection)	Not stated ("technical concentrate")	Negative	Chruscielska et al. (2000)
Cytogenetic damage[c]	Sprague-Dawley rats, bone marrow. Sampling after 6 h, 12 h and 24 h	0–1000 mg/kg bw (single intraperitoneal injection)	98.7	Negative	Li (1983b, 1983c) (published by Li & Long, 1988)
Dominant lethal mutation[c]	Charles River CD-1 mice, males treated and paired with a total of 16 untreated dams over a period of 8 weeks	0, 200, 800 or 2000 mg/kg bw (single oral dose)	98.7	Negative	Rodwell (1980)
Alkaline elution assay for DNA single-strand breaks and formation of alkali-labile sites[d]	Liver and kidney of male Swiss CD-1 mice. Sampling 4 h and 24 h after administration	0 and 300 mg/kg bw (single intraperitoneal administration)	99.9	Weakly positive[d] after 4 h in both organs, suggesting possible transient DNA damage. Biological significance equivocal, effects might also be due to toxicity.	Bolognesi et al. (1997)

Table 26. Continued

End-point	Test object	Concentration	Purity (%)	Result	Reference
Oxidative DNA damage measured by quantification of 8-hydroxydes-oxyguanosine (8-OhdG) adducts	Liver and kidney of Swiss CD-1 mice. Sampling after 8 h and 24 h after dosing.	0 and 300 mg/kg, (single intraperitoneal administration)	99.9	Positive (increase in 8-OhdG adducts in the liver after 24 h). The promutagenic DNA lesion 8-OhdG is a biomarker for oxidative stress.	Bolognesi et al. (1997)
Measurement of DNA adducts using ^{32}P-postlabelling technique	Liver and kidney of Swiss CD-1 mice	0, 130, 270 mg/kg bw, (single intraperitoneal administration)	Glyphosate IPA salt, no details of purity given	Negative (no increase in relative level of adducts)	Peluso et al. (1998)
Wing-spot[e]	*Drosophila melanogaster* larvae	0.1, 0.5, 1, 2, 5, 10 mmol/l in distilled water	96	Weakly positive only in the standard crosses	Kaya et al. (2000)

[a] A positive control was included; GLP and QA statements were included. Complied with current regulatory guidelines
[b] Study complied with GLP and is considered to be acceptable, QA statements were included
[c] The study was performed before GLP but is considered to be acceptable
[d] The use of the intraperitoneal route of administration when the liver is to be sampled is inappropriate when an assessment of the in-vivo status is required, since the deposition of test material into the intraperitoneal cavity gives, in effect, an in-vitro exposure. This assay is a non-selective measurement of the migration of DNA through a filter and is a measurement of DNA size. Any factor that affects the DNA size is therefore detectable. One of the most important elements for control is that of toxicity. If the materials (or the manipulative procedures) induce cytotoxicity in the population under investigation, then the result will be an increase in the elution rate constant. Therefore, this assay cannot distinguish between toxicity-induced DNA damage and genotoxicity-induced DNA damage. The lack of reported controls, with the choice of administration route, makes it difficult to draw any conclusions from these data
[e] No information was provided on toxicity after treatment

2.5 Reproductive toxicity

(a) Multigeneration studies

In a two-generation study conducted in compliance with the principles of GLP and according to the guidelines of the US EPA and the OECD (TG 416), groups of 28 male and 28 female Crl:CD(SD)BR VAF/Plus rats (aged 6 weeks at the start of treatment) were fed diets containing glyphosate technical (purity, 99.2%) at a concentration of 0, 1000, 3000 or 10 000 ppm for 70 days before their first mating and until termination. The highest dietary concentraion was set at 10 000 ppm since administration of diets containing glyphosate at 30 000 ppm in a preliminary study was associated with signs of maternal toxicity. The F_1 generation (24 males and 24 females per group) was selected from the F_{1A} litters and treated from 1 week after weaning for at least 84 days before first mating. Each generation was mated twice, changing partners for the second mating and avoiding sister/brother matings throughout. Treatment was continued for both sexes until the day 21 of weaning of the second litter when animals were sacrificed for organ weighing, gross pathological examination and microscopy of reproductive tissues parents of both generations in the control group and at the highest dose. On postnatal day 4, litters were adjusted (as far as possible) to four male and four female pups. Fresh diets were prepared weekly and were appropriately controlled for concentration, homogeneity and stability on several occasions throughout the study. The overall calculated mean daily intake of glyphosate during the pre-mating phase was 0, 66, 197 and 668 mg/kg bw per day for F_0 males; 0, 75, 226 and 752 mg/kg bw per day for F_0 females; 0, 76, 230 and 771 mg/kg bw per day for F_1 males; and 0, 82, 245 and 841 mg/kg bw per day for F_1 females.

In adults, parameters studied were signs of reaction to treatment, mortality, food and water consumption, body-weight changes, mating performance and pregnancy rate, length of gestation, weighing of relevant organs (approximately eight), preservation of tissues (approximately 40) after macroscopic examination of respective organs, including microscopy of salivary glands in all surviving F_0 and F_1 animals. Litter data comprised number and state of pups at parturition, sexing, weighing and examination for external abnormalities. Internal abnormalities were studied in pups culled by postnatal day 4. Also recorded were the onset of vaginal opening and cleavage of the balanopreputial skinfold (F_1 generation only).

No treatment-related clinical signs were noted in the parents of either generation. There was a total of four mortalities in each parent generation; however, none of the mortalities were considered to be treatment-related. The highest dose caused a slight increase in food and water consumption of F_1 females, a slightly lower mean body weight of F_1 males at selection for the second generation, but a weight gain comparable to that of controls from this point. There were no adverse effects of treatment on mating performance, pregnancy rate or duration of pregnancy in either generation. There were no effects on the total number of litters being born within groups, total litter loss, litter size, pup mortality or sex ratio. Litter weights in all treated groups were lower at the first F_0 mating; however, this was not seen at the second F_0 mating or in either F_1 mating, so it is not considered to be an adverse effect of treatment with glyphosate. There was no effect on sexual maturation in either sex as evaluated by mean age at vaginal opening or attainment of balanopreputial skin-fold cleavage in female or males respectively.

Treatment-related histopathological changes were apparent in the parotid salivary gland of both F_0 and F_1 males and females at 3000 ppm and at 10 000 ppm, and in the submaxillary salivary gland of F_0 females at 3000 ppm and at 10 000 ppm, and F_1 females at 10 000 ppm (Table 27). The changes manifested as hypertrophy of acinar glands with prominent granular cytoplasm, the morphology severity was classified as "minimal" (grade 2) on a scale from "trace" (grade 1) to "severe" (grade 5). There were no other treatment-related macroscopic or histopathological findings in adult rats or offspring, no effects on any organ weights (including reproductive organs).

In conclusion, administration of glyphosate at a dietary concentration of up to 10 000 ppm and over two successive generations had no effect on sexuality and fertility of

Table 27. Incidence of cellular alteration of salivary glands in a multigeneration study in rats fed diets containing glyphosate

Alteration	Dietary concentration (ppm)							
	Males				Females			
	0	1000	3000	10 000	0	1000	3000	10 000
F_0 generation:								
Parotid gland	2/27	2/28	3/28	12/26	0/28	2/27	5/28	17/28
Submaxillary gland	0/27	—	—	0/26	0/28	1/27	4/28	14/28
F_1 generation:								
Parotid gland	1/24	0/24	4/23	10/23	0/24	0/27	4/24	9/23
Submaxillary gland	0/24	—	—	0/23	0/24	0/27	0/24	3/23

From Brooker et al. (1992)
—, not examined
No statistical analysis was done

males or females. The NOAEL for parental and offspring toxicity was 3000 ppm, equal to 197 mg/kg bw per day, on the basis of increased food and water consumption of F_1 females, lower body weight of F_1 males, and an increased incidence of cellular alteration of the parotid (males and females) and submaxillary (females only) salivary glands in both F_0 and F_1 adults at 10 000 ppm (Brooker et al., 1992).

In a two-generation study conducted in compliance with the principles of GLP and according to the guidelines of the US EPA and the OECD (TG 416), groups of 26 male and 26 female Wistar-derived Alpk:AP$_f$SD rats (aged 5–6 weeks at the start of treatment) were fed diets containing glyphosate technical (purity, 97.6%) at a concentration of 0, 1000, 3000 or 10 000 ppm. After 10 weeks, the animals were mated and allowed to rear the ensuing F_{1A} litters to weaning. The breeding programme was repeated with the F_1 parents selected from the F_{1A} offspring to produce the F_{2A} litters after a 10-week pre-mating period. Diets were appropriately controlled for concentration, homogeneity and stability on several occasions throughout the study. The overall calculated mean daily intake of glyphosate during the pre-mating phase was 0, 99, 293 and 985 mg/kg bw per day for F_0 males; 0, 104, 323 and 1054 mg/kg bw per day for F_0 females; 0, 117, 352 and 1161 mg/kg bw per day for F_1 males; and 0, 123, 371 and 1218 mg/kg bw per day for F_1 females.

Observations and measurements in adults comprised clinical observations, food and water consumption, body-weight changes, reproductive performance, estrous cycle, developmental landmarks (F_1 only), and post-mortem examinations, including uterine assessment, organ weights, sperm analysis, histopathology and quantification of oocytes (F_1 only). Observations and measurements for pups comprised number at birth until day 29, survival, individual and litter weight, clinical condition, sex distribution, and post-mortem examination including organ weights of selected pups.

There were no treatment-related mortalities or clinical findings in parents of either generation. The effects of glyphosate on body weight and food consumption were confined to the F1 males given 10 000 ppm, with a statistically significantly lower body weight from week 2 to week 8 and a statistically significantly lower food consumption throughout the pre-mating period (Table 28). Food utilization values over the duration of the study were not statistically significantly different from those of the controls.

Table 28. Body weights (adjusted for initial weight) and food consumption during the pre-mating period for F_1 males fed diets containing glyphosate

Parameter	Dietary concentration (ppm)			
	0	1000	3000	10 000
Body weight(g)				
Week 1	80.2	81.1	78.1	75.3
Week 4	246.2	247.6	242.8	237.3**
Week 8	403.6	410.1	395.3	387.0*
Week 11	461.7	471.3	455.5	449.7
Food consumption (g/rat per day)				
Week 1	19.3	19.7	19.0	18.1*
Week 4	34.6	35.5	33.9	32.6**
Week 8	35.5	36.1	34.1	33.0**
Week 10	35.5	35.7	34.1	33.0**

*$p < 0.05$, **$p < 0.01$; Student's t-test, two-sided
From Moxon (2000)

Table 29. Adjusted mean body weights (g) of F_{1A} and F_{2A} pups

Litter	Dietary concentration (ppm)							
	Males				Females			
	0	1000	3000	10 000	0	1000	3000	10 000
F_{1A} pups								
Day 1	5.8	6.1	6.0	6.1	5.4	5.8	5.6	5.7
Day 5	9.2	9.1	8.9	8.5	9.0	8.5	8.4	8.1**
Day 8	13.8	13.4	13.2	12.6*	13.3	12.8	12.4	12.1**
Day 15	26.8	26.1	25.8	24.6*	26.1	25.2	24.5	23.8*
Day 22	43.4	42.4	41.4	39.2*	41.9	40.3	39.4	37.7*
Day 29	81.7	79.5	79.6	74.6*	77.1	74.0	74.1	69.9**
F_{2A} pups								
Day 1	6.3	6.3	6.3	6.2	6.1	5.9	5.9	5.8
Day 5	9.7	9.9	9.3	9.5	9.3	9.6	9.1	9.1
Day 8	14.3	14.7	13.8	14.2	13.8	14.2	13.4	13.7
Day 15	27.4	28.3	26.4	27.5	26.7	27.5	25.8	26.5
Day 22	44.5	46.2	43.1	44.9	42.7	44.8	41.8	42.9
Day 29	83.0	86.0	80.6	82.8	77.7	80.6	75.6	77.4

*$p < 0.05$, **$p < 0.01$; Student's t-test, two-sided
From Moxon (2000)

Glyphosate did not have an adverse effect on the estrous cycle in females, on the number of primordial follicles in F_1 females, or on the number of sperm, sperm motility parameters or morphology in males, or on reproductive performance in either sex in either generation. There was no adverse effect of glyphosate on developmental landmarks (time to preputial separation or vaginal opening) or pup survival, on litter size during lactation, on the clinical condition of the pups or on the proportion of male pups in either the F_{1A} or F_{2A} litters. The body weights of F_{1A} pups were lower in comparison to those in the control group from day 8 onwards, but a similar effect was not seen in the F_{2A} pups (Table 29). There was no treatment-related effect on total litter weight.

At sacrifice, liver and kidney weights adjusted for body weight of F_0 males at 10 000 ppm were slightly but statistically significantly higher (about 5 and 4%, respectively) than concurrent control values. Similar changes were not observed in the F_1 males or in adult females of either generation. No histopathological changes were observed in any tissue from the F_0 or F_1 animals that could be attributed to treatment.

In conclusion, administration of glyphosate at a dietary concentration of up to 10 000 ppm and over two successive generations had no effect on the sexuality or fertility of males and females. The NOAEL for parental and offspring toxicity was considered to be 3000 ppm, equal to 293 mg/kg bw per day, on the basis of a reduction in body weight of F_{1A} pups and a subsequent reduction in body weight of F_1 parent males at 10 000 ppm (Moxon, 2000).

(b) Developmental toxicity

Rats

In a study of developmental toxicity conducted in compliance with the principles of GLP and according to the guidelines of the US EPA and the OECD (TG 414), groups of 25 time-mated female Crl:CD(SD)BR VAF/Plus rats were given glyphosate (purity, 98.6%; in aqueous solution/suspension with 1% methylcellulose) at a dose of 0, 300, 1000 or 3500 mg/kg bw per day by gavage on days 6–15 of gestation (day 0 being the day of mating).

All animals were observed daily for clinical signs and mortality, and body weight and food consumption were measured on days 1, 3, 8, 10, 12, 14, 16, 18 and 20 of gestation. Water consumption was measured daily. On day 20 of gestation, the dams were killed, and a macroscopic examination was carried out post mortem. Pregnancy status was determined and numbers of corpora lutea, live fetuses and intrauterine deaths were recorded. All live fetuses were weighed, examined for external abnormalities, and sexed by gonadal inspection. Approximately half the fetuses in each litter were prepared and examined for skeletal alterations (modified Dawson technique), and the remainder were prepared and examined for soft tissue alterations (Wilson technique).

There were two maternal deaths at the highest dose after signs of respiratory distress on day 7 and 13, respectively, and another dam at the highest dose was sacrificed on day 10 after a probable intubation error. At the highest dose, clinical abnormalities included salivation, loose stools and noisy respiration. The latter was also observed in two animals at the intermediate dose on one occasion. Body-weight gain was markedly reduced at the highest dose (by 16–81% of control values, days 6–20 of gestation) and marginally reduced at the intermediate dose (by 86–97% of control values, days 6–20 of gestation). Food consumption was slightly decreased at the highest dose during the dosing period (75–94% of control values, days 6–15 of gestation), but was comparable with controls thereafter. Water intake was increased at the highest dose (139–205% of control values, days 6–15 of gestation). No treatment-related changes were observed at any dose at necropsy.

A total of 23, 23, 25 and 22 dams had live young at day 20 in the control group, and at the lowest, intermediate and highest dose, respectively. There was no significant influence of treatment on embryonic losses, litter size or sex ratio, but the litter weights and mean fetal weights were reduced at the highest dose, the latter being statistically significant (90% and 94% of control values, respectively). The occurrence of malformations was not significantly increased by treatment. However, the incidence of rib distortion (wavy ribs) was markedly higher at the highest dose and slightly higher at the intermediate dose; the incidences on the basis of fetuses (litters) were 1 (1), 0 (0), 3 (2), and 28 (11) for the control group, at the lowest, intermediate and highest dose, respectively. In addition, reduced ossification was seen slightly more frequently at the highest and intermediate doses. As result, the percentage of fetuses showing skeletal anomalies (variations) was significantly increased at the two higher doses, but the percentage of fetuses affected at the intermediate dose exceeded the historical background range (21.9–27.2%) only slightly (Table 30).

The NOAEL for maternal toxicity was 300 mg/kg per day on the basis of clinical signs and reduced body-weight gain at 1000 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 300 mg/kg per day on the basis of an increased incidence of delayed

Table 30. Incidence of fetal skeletal anomalies in a study of developmental toxicity in rats given glyphosate by gavage

	Dose (mg/kg bw per day)			
	0	300	1000	3500
No. of fetuses (litters) examined	155 (23)	143 (23)	166 (25)	142[a] (22)
No. of fetuses (litters) affected	19 (11)	36 (16)	46 (19)	55 (19)
Mean (% of fetuses)	11.7	22.6	28.4*	35.7**

From Brooker et al. (1991b)

*$p < 0.05$, **$p < 0.01$; Kruskal-Wallis test, and distribution-free Williams' test

[a] Two malformed fetuses were excluded

ossification and an increased incidence of fetuses with skeletal anomalies at 1000 mg/kg bw per day and greater (Brooker et al., 1991b).

In a study of developmental toxicity conducted in compliance with the principles of GLP and according to the OECD Guidelines for Testing of Chemicals No. 414, groups of 24 time-mated female Alpk:APfSD (Wistar-derived) rats were given glyphosate (purity, 95.6%; in deionized water) at a dose of 0, 250, 500 or 1000 mg/kg bw per day by gavage on days 7–16 of gestation (day 1 being the day of mating). The animals were observed routinely for physical appearance, behaviour, body-weight gain and food consumption. On day 22 of gestation, the dams were killed, and a macroscopic examination carried out post mortem. Pregnancy status was determined and numbers of corpora lutea, live fetuses and intrauterine deaths recorded. All fetuses were weighed, examined for external and visceral abnormalities, sexed, eviscerated and fixed, and sections of the head were examined for abnormalities of the brain. The carcasses were then prepared and examined for skeletal alterations.

One control animal was killed on day 7 as a result of mis-dosing; there were no other mortalities. There were no changes in the clinical condition of the dams given glyphosate that were considered to be treatment-related, and there was no effect on body weight, food consumption or macroscopic findings post mortem. There was no evidence of developmental toxicity attributable to glyphosate as assessed by the number, growth or survival of the fetuses. Observation of the external appearance of the fetuses, examination of the viscera and assessment of the skeletons revealed no treatment-related findings.

The NOAEL for both maternal and developmental effects was 1000 mg/kg bw per day, the highest dose tested (Moxon, 1996a).

Rabbits

In a study of developmental toxicity conducted in compliance with the principles of GLP and according to the OECD Guidelines for Testing of Chemicals No. 414, groups of 16–20 time-mated female New Zealand White rabbits were given glyphosate (purity, 98.6%; in aqueous solution/suspension with 1% methylcellulose) at a dose of 0, 50, 150 or 450 mg/kg bw per day by gavage on days 7–19 of gestation (day 0 being the day of mating). Dosage volumes were calculated for individual animals on day 7 of gestation and adjusted according to body weight on days 9, 11 and 15. All animals were observed daily for clinical signs and mortality, and body weight and food consumption were measured on days 1, 7, 9, 11, 15, 20, 24 and 29 of gestation. On day 29 of gestation, the dams were killed, and a macroscopic examination post mortem was carried out. Pregnancy status was determined and numbers of corpora lutea, live fetuses and intrauterine deaths were recorded. All live fetuses were examined for external abnormalities, weighed, and prepared and examined for soft tissue abnormalities and for skeletal abnormalities (modified Dawson technique). Where appropriate, abnormalities were examined by additional procedures (e.g. microdissection, histopathology) to clarify initial observations.

One animal at the highest dose was found dead on day 20 of gestation after signs of abortion on day 19 of gestation, gastrointestinal disturbances, and a severe reduction in food intake and body-weight loss from the start of treatment. There was a dose-related increase in the incidence of females with soft/liquid faeces and inappetence (lack of appetite) at the intermediate and highest doses. Also, food consumption was slightly reduced at the

intermediate dose (by 88–89% of the value for controls, days 11–19 of gestation) and at the highest dose (by 83–90% of the value for controls, days 9–19 of gestation), while body-weight gain at these doses was 80% and 67% of control value (days 7–20 of gestation), respectively. No treatment-related changes were observed at any dose at necropsy.

There were 18, 12, 15 and 13 viable litters in the control group and at the lowest, intermediate, and highest doses, respectively. Pre-treatment events (corpora lutea, pre-implantation loss) showed no significant differences between groups. In the treated groups, there was a significant increase in the number of embryonic deaths per litter and, hence, in postimplantation loss when compared with these values in the concurrent control group, although no clear dose–response relationship was evident (Table 31). Consequently, litter size and litter weight showed a dose-related reduction in all treated groups (not statistically significant). No adverse effect of treatment was noted for mean fetal weight.

A total of three (three), three (three), five (three) and six (five) fetuses (litters) out of 163, 104, 112 and 95 fetuses examined showed malformations in the control group and at the lowest, intermediate and highest dose, respectively. The slightly higher number of fetuses with malformations at the intermediate and highest dose was caused by an apparent increase in the incidence of fetuses with interventricular septal defect and other abnormalities affecting the heart—the number of fetuses affected in the control group and at the lowest, intermediate and highest doses being one, one, four and five, respectively. The mean percentage of malformed fetuses per litter, however, was within the concurrent background range in all groups (13 studies performed in 1989; mean incidence of 3.8 with a range of 0.7 to 5.9).

The NOAEL for maternal toxicity was 50 mg/kg per day on the basis of clinical signs and reduced food consumption and body-weight gain at 150 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 150 mg/kg per day on the basis of a slightly increased incidence of late embryonic deaths and postimplantation loss at 450 mg/kg bw per day (Brooker et al., 1991a).

In a study of developmental toxicity conducted in compliance with the principles of GLP and according to the OECD Guidelines for Testing of Chemicals No. 414, groups of 20 time-mated female New Zealand White rabbits were given glyphosate (purity, 95.6%; in deionized water) at a dose of 0, 100, 175 or 300 mg/kg bw per day by gavage on days 8–20 of gestation (day 1 being the day of mating). Dosage volumes were calculated for

Table 31. Incidence of embryonic deaths in a study of developmental toxicity in rabbits given glyphosate by gavage

Parameter (mean No.)	Dose (mg/kg bw per day)				Range for historical controls (mean)[a]
	0	50	150	450	
Implantations	9.7	10.5	9.0	9.2	7.0–11.1 (9.5)
Early embryonic deaths	0.4	0.9	0.9	0.5	0.3–1.1 (0.6)
Late embryonic deaths	0.2	0.9	0.5	1.3**	0.1–1.3 (0.7)
Abortions	0	0	0.1	0	0–0.1 (0)
Total embryonic deaths	0.6	1.8*	1.5*	1.8*	0.6–2.0 (1.2)
Postimplantation loss (%)	5.7	19.5*	15.3*	21.0**	6.5–17.5 (12.9)
Live young	9.1	8.7	7.7	7.3	6.1–9.5 (8.2)

From Brooker et al. (1991a)

*$p < 0.05$, **$p < 0.01$; Kruskal-Wallis test, and distribution-free Williams' test

[a] From 21 studies performed between January 1989 and June 1990

individual animals according to their daily body weights. All animals were observed daily for clinical signs and mortality, while body weight and food consumption were measured on days 1, 4, 8–20, 23, 26 and 30 of gestation and on days 8, 11, 14, 17, 20, 23, 26 and 30 of gestation, respectively. On day 30 of gestation, the dams were killed, and a macroscopic examination was carried out post mortem. Pregnancy status was determined and numbers of corpora lutea, implantations, live fetuses and intrauterine deaths were recorded. All fetuses were examined for external abnormalities, weighed, and prepared and examined for soft tissue abnormalities and for skeletal abnormalities (modified Dawson technique). Additionally, assessment of ossification including scoring of *manus* and *pes* was performed.

The incidence of intercurrent maternal deaths was 1, 2, 2 and 2 in the control group, and at the lowest, intermediate and highest dose, respectively. There was a dose-related increase in the incidence of dams with signs of diarrhoea and reduced faecal output at the intermediate and highest doses. Food consumption was significantly reduced at the intermediate dose (by 72–86% of the value for controls, days 8–20 of gestation) and the highest dose (by 57–81% of the value for controls, days 8–20 of gestation), while body-weight gain at these doses was 70% and 38% of the value for controls (days 8–20 of gestation), respectively. No treatment-related changes were observed at any dose at necropsy.

There were 17, 18, 17 and 17 viable litters in the control group and at the lowest, intermediate and highest dose, respectively. The mean fetal weight (44.4, 43.3, 43.2 and 40.7 g for the control group and at the lowest, intermediate and highest doses, respectively) was statistically significantly reduced at the highest dose, which was attributed to the occurrence of two litters for which the mean fetal weight was particularly low (20.3 g and 29.6 g). There was no effect of treatment on the number or survival of the fetuses in utero. The number of fetuses with major defects was 3 out of 143, 1 out of 147, 0 out of 135 and 2 out of 144 in the control group and at the lowest, intermediate, and highest dose, respectively. Neither the type nor incidence of major defects indicated a treatment-related effect. The proportion of fetuses with minor skeletal defects was statistically significantly increased at the lowest and highest doses, when compared with that in the control group, but not at the intermediate dose. Consideration of the specific defects revealed a statistically significantly increased incidence of fetuses with partially ossified transverse processes of the seventh vertebra in the group receiving the highest dose (5.6%, compared with 0.7% in controls), unossified transverse processes of the seventh lumbar vertebra (9.7%, compared with 2.8% in controls) or partially ossified sixth sternebra (11.1%, compared with 2.8% in controls). Owing to the reduction in ossification, at the highest dose the mean *manus* score per litter (3.05, compared with 2.88 in controls) and the mean *pes* score per litter (1.18, compared with 1.07 in controls) were slightly increased.

The NOAEL for maternal toxicity was 100 mg/kg per day on the basis of clinical signs and reduced food consumption and body-weight gain at 175 mg/kg bw per day and greater. The NOAEL for developmental toxicity was 175 mg/kg per day on the basis of reduced fetal weight and reduced ossification at 300 mg/kg bw per day (Moxon, 1996b).

2.6 Special studies

(a) Neurotoxicity

In a study of acute neurotoxicity conducted in compliance with the principles of GLP and according to OECD guideline 424, groups of 10 male and 10 female Alpk:AP$_f$SD rats were given glyphosate (purity, 95.6%; in deionized water) as a single dose at 0, 500, 1000

or 2000 mg/kg bw by gavage and sacrificed 2 weeks later. All animals were observed before the start of the study and daily throughout the study for changes in clinical condition. Detailed clinical observations including qualitative assessments of landing foot splay, sensory perception and muscle weakness were performed at weekly intervals. Locomotor activity was also monitored at weekly intervals. Body weights and food consumption were measured throughout the study. At the end of the study, five rats of each sex per group were sacrificed and subjected to whole-body perfusion fixation. Selected nervous system tissues including brain (seven levels including the cerebral cortex, hippocampus, cerebellum, pons and medulla), spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic, sural and tibial nerves removed and processed for microscopic examination. Brains were weighed and measured (length and width). Histopathological examination was performed on animals in the control group and at the highest dose only.

Administration of glyphosate produced clinical signs of toxicity (including decreased activity, subdued behaviour, hunched posture, sides pinched in, tip-toe gait and/or hypothermia) at approximately 6 h after dosing in 3 out of 10 females at 2000 mg/kg bw. One of these females was found dead on day 2 of the study. The clinical signs seen were considered to reflect general toxicity attributable to treatment with glyphosate. One female dosed at 500 mg/kg bw was found dead approximately 6 h after dosing on day 1, but in the absence of any treatment-related clinical signs, this death was considered not to be treatment-related. There were no treatment-related clinical observations at 500 or 1000 mg/kg bw in either sex or in males at 2000 mg/kg bw. Mean food consumption at 2000 mg/kg bw was slightly reduced for females (92% of value for controls; $p < 0.05$) and males (95% of value for controls; not significant) during week 1, while body weights were not affected at any dose. There were no treatment-related changes in the FOB, landing foot splay, sensory perception, grip strength or motor activity. At necropsy, no treatment-related macroscopic changes and no effects on brain weight, length or width were observed. Histopathological evaluation of the central and peripheral nervous system revealed no treatment-related changes in animals receiving glyphosate at a dose of 2000 mg/kg bw.

The NOAEL for neurotoxicity was 2000 mg/kg bw, the highest dose tested. The NOAEL for general toxicity was 1000 mg/kg bw on the basis of lethality and general clinical signs of toxicity at 2000 mg/kg bw (Horner, 1996a).

In a short-term study of neurotoxicity that was conducted in compliance with the principles of GLP and according to OECD guideline 424, groups of 12 male and 12 female Alpk:AP$_f$SD rats were fed diets containing glyphosate (purity, 95.6%) at a concentration of 0, 2000, 8000 or 20 000 ppm for 13 weeks. Diets were appropriately controlled for concentration, homogeneity and stability at regularly intervals throughout the study. The overall calculated mean daily intake of glyphosate was 156, 617 and 1547 mg/kg bw per day for males and 166, 672 and 1631 mg/kg bw per day for females. All animals were observed before the start of the study and daily throughout the study for changes in clinical condition and behaviour. Detailed clinical observations, including qualitative assessments of landing foot splay, sensory perception and muscle weakness, were performed at intervals during the study. Locomotor activity was also monitored at intervals. Body weights and food consumption were measured throughout the study. At the end of the study, six rats of each sex (which had been pre-designated for neuropathology) per group were sacrificed and subjected to whole-body perfusion fixation. Selected nervous system tissues including brain (seven levels including the cerebral cortex, hippocampus, cerebellum, pons and medulla),

spinal cord (cervical and lumbar), Gasserian ganglion, dorsal root ganglia and spinal roots (cervical and lumbar), gastrocnemius muscle, sciatic, sural and tibial nerves were removed and processed for microscopic examination. Brains were weighed and measured (length and width). Histopathological examination was performed on animals in the control group and at the highest dose only. At termination, all animals not required for neuropathology were killed and discarded.

Administration of glyphosate resulted in treatment-related reductions in growth and food utilization for males fed diets containing glyphosate at 20 000 ppm, with no associated effects on food consumption (Table 32). There were no treatment-related effects on body weight, food consumption or food utilization for males fed 2000 or 8000 ppm glyphosate, or for females at any dose.

There were no clinical signs of toxicity or effects on any of the quantitative functional observation battery tests or on locomotor activity that indicated any neurotoxic potential. At necropsy, no treatment-related macroscopic changes and no effects on brain weight, length or width were observed. Histopathological evaluation of the central and peripheral nervous system revealed no treatment-related changes in animals dosed with glyphosate at a dietary concentration of 20 000 ppm.

The NOAEL for neurotoxicity was 20 000 ppm, equal to 1547 mg/kg bw per day, the highest dose tested. The NOAEL for general toxicity was 8000 ppm, equal to 617 mg/kg bw per day, on the basis of reduced growth and reductions in food utilization in male rats at 20 000 ppm (Horner, 1996b).

In a study of acute delayed neurotoxicity conducted in compliance with the principles of GLP and according to OECD guideline 418, 20 hens (hybrid brown laying strain—Lohmann Brown) were given a single oral dose of glyphosate (purity, 95.6%) at 2000 mg/kg bw. In addition, two groups of 12 hens were dosed with distilled water or tri-ortho-cresyl phosphate (TOCP) at a dose of 1000 mg/kg bw and served as negative and positive controls, respectively. Observations in the following 21/22 days included mortality, clinical signs, assessment of delayed locomotor ataxia and body weight. Measurements of brain acetylcholinesterase, and neuropathy target esterase in the brain and lumbar spine were made for three hens from each treatment group, 48 h after dosing. At the end of the obser-

Table 32. Body weights and food utilization in rats fed diets containing glyphosate for 13 weeks

	Dietary concentration (ppm)							
	Males				Females			
	0	2000	8000	20 000	0	2000	8000	20 000
Body weights (g)								
Week 1	216.0	217.0	218.6	215.0	173.5	178.8	175.6	175.3
Week 4	338.2	340.7	339.6	323.7*	214.3	228.3**	224.9**	219.2
Week 8	440.7	440.1	429.1	405.8**	253.6	262.1	260.4	255.4
Week 12	510.3	506.8	497.8	471.1**	278.9	288.2	279.8	276.0
Week 14	534.7	532.8	526.5	496.1**	285.1	291.5	287.9	281.0
Food utilization (g of growth/100 g of food)								
Weeks 1–4	18.13	17.16	16.94	16.28*	9.42	9.73	9.36	9.61
Weeks 5–8	11.52	10.69	10.35	9.93*	5.99	5.55	5.39	5.70
Weeks 1–13	12.00	11.45	11.38	10.87**	6.08	6.03	6.06	5.96

From Horner (1996b)
* $p < 0.05$, ** $p < 0.01$; Student's t-test, two-sided

vation period, six hens from each treatment group were selected for histopathological examination of the forebrain, mid- and hindbrain, upper cervical, lower cervical, mid-thoracic and lumbo-sacral spinal cord, proximal sciatic nerve, distal sciatic nerve and tibial nerve.

There was no evidence of clinical ataxia in any of the negative controls or in any of the hens dosed with glyphosate. Five of the hens dosed with TOCP developed clinical ataxia, starting between days 11 and 21. There was no effect on body weights for hens dosed with glyphosate, but hens dosed with TOCP showed an overall weight loss. Acetylcholinesterase activity in brain samples was reduced by 19% in hens treated with TOCP. It was reduced by 6% in hens treated with glyphosate, but was not statistically significant and was considered of no toxicological significance. There was no effect on neuropathy target esterase activity in brain or spinal cord for the hens treated with glyphosate, but in the positive controls there was an 84% and 78% reduction in brain and spinal cord neuropathy target esterase activities, respectively, compared with the negative controls. At necropsy, no macroscopic abnormalities were seen in any of the hens examined. Histopathological examination revealed no evidence of acute delayed neurotoxicity or any other treatment-related changes in hens treated with glyphosate. Hens treated with TOCP showed significant axonal degeneration in spinal cord, peripheral nerve and cerebellum, demonstrating the validity of the test system.

The NOAEL for acute delayed neurotoxicity of glyphosate in hens was 2000 mg/kg bw (Johnson, 1996).

In a non-guideline experiment, a cell culture model was used to determine if chronic exposure to organophosphate pesticides can alter the sensitivity of nerve cells to subsequent acute exposure to organophosphates or other compounds. NB2a neuroblastoma cells were grown in the presence of diazinon at a concentration of 25 µmol/l for 8 weeks. The organophosphate was then withdrawn and the cells were induced to differentiate in the presence of various other pesticides, including glyphosate (purity, >99%). The resulting outgrowth of neurite-like structures was measured by light microscopy and quantitative image analysis and the IC_{50} for each organophosphate or formulation was calculated. The IC_{50} values in diazinon-pre-exposed cells were compared with the equivalent values in cells not pre-exposed to diazinon. The IC_{50} for inhibition of neurite outgrowth by acute application of diazinon, pyrethrum, glyphosate or a commercial formulation of glyphosate was decreased by between 20% and 90% after pre-treatment with diazinon. According to the study authors, the data support the view that long-term exposure to an organophosphate may reduce the threshold for toxicity of some environmental agents (Axelrad et al., 2003).

(b) Mechanism of induction of salivary gland changes

In a study of the mechanism of induction of salivary gland lesions performed by the United States National Toxicology Program (NTP), two groups of four male F344/N rats were fed diets containing glyphosate (purity, 99%) at a concentration of 50 000 ppm (which was the highest dose used in a short-term study on toxicity), together with continuous subcutaneous infusion of propanolol (a β-blocker; 1.2 mg/kg bw per day) or a vehicle (water). Three additional groups of four male rats received control diet, together with continuous subcutaneous infusion of isoproterenol (a β-adrenergic agonist; 1.0 mg/kg bw per day), isoproterenol plus propanolol, or a vehicle (water). After 14 days of treatment, the animals were sacrificed and the parotid and submandibular/sublingual glands were removed, weighed and processed for electron and light microscopy.

All rats survived to the end of the study. Rats receiving isoproterenol were hypoactive and had increased respiratory rates on day 1, but were normal by the following day. While there was no effect on food consumption in any group, there was a significant decrease in body-weight gains in the groups that received glyphosate (6.3 g and 6.0 g compared with 16.0 g in controls). Both glyphosate and isoproterenol produced increased salivary gland weights, with the parotid gland being more affected (280% or 154% of weights in the control group for glyphosate or isoproterenol, respectively). When both compounds were given along with propanolol, parotid weights were 194% of those of the controls for glyphosate but only 109% of those of the controls for isoproterenol. In the parotid and in the submandibular gland, increased weights were associated with cytoplasmic changes of acinar cells (basophilic change, fine vacuolation, swelling, loss of the normal periodic acid–Schiff (PAS)-positive reactivity of the secretory granules). The study authors concluded that the salivary gland effects induced by glyphosate were mediated through an adrenergic mechanism (Chan & Mahler, 1992).

In a study conducted in compliance with the principles of GLP and designed for comparison of salivary gland effects in three strains of rats, groups of 24 male Alpk:ApfSD (Wistar-derived) (AP), Sprague-Dawley (Charles River CD) (CD), and Fischer 344 rats (F344) were fed diets containing glyphosate (purity, 95.6%) at a concentration of 0 (control) or 20 000 ppm (equivalent to approximately 2000 mg/kg bw per day) for 28 days. Eight animals from each group were killed on day 29 and the remaining animals were retained on control diet for a further 4 weeks (eight rats per group) or 13 weeks (eight rats per group). Clinical observations, body weights and food consumption were measured, and at the end of the scheduled periods, the animals were killed and subjected to a gross examination of the salivary glands. The salivary glands were weighed, and the left salivary glands were taken for microscopic examination.

Treatment with glyphosate at 20 000 ppm produced significant reductions in body weight and minor reductions in food consumption in AP and CD rats, but no effects were seen in F344 rats. In contrast, weight of salivary glands was unaffected in CD rats, but was increased in AP and F344 rats at the end of the 4 weeks. Microscopic examination of the salivary gland showed that the most pronounced effect occurred in F344 rats, where there was diffuse cytoplasmic basophilia and enlargement of the parotid acinar cells. Similar but slight effects involving small foci of cells only occurred in the AP and CD rats.

Recovery from effects was apparent in all strains during the recovery periods. Body weight and food consumption returned to control values in both AP and CD rats. After 4 weeks on control diet, significant recovery of the salivary gland changes, in terms of both weight and histopathology, was evident in the F344 rats, and the AP and CD rats were indistinguishable from their corresponding controls. After 13 weeks on control diet, more F344 rats treated with glyphosate showed minor focal changes in the salivary gland compared with the respective controls, and group mean weights of the salivary gland were increased slightly (Table 33).

In conclusion, administration of diets containing glyphosate at a concentration of 20 000 ppm (equivalent to approximately 2000 mg/kg bw per day) to male rats for 4 weeks produced minor strain differences in systemic toxicity (changes in body weight and food consumption) and marked strain differences in the severity of effects in the parotid salivary gland. The most pronounced effects in the salivary glands were seen in F344 rats and the

Table 33. Selected findings in three strains of male rats given diets containing glyphosate for 28 days

Finding	Dietary concentration (ppm)					
	AP rats		CD rats		F344 rats	
	0	20 000	0	20 000	0	20 000
Body weight (g) at termination						
After 4 weeks of treatment	353.4	344.9	379.9	346.4	213.9	209.5
After 4 weeks of recovery	471.1	428.4	462.4	424.0	254.0	265.6
After 13 weeks of recovery	523.1	518.9	514.1	534.4	336.0	325.5
Salivary gland weight (mg)[a]						
After 4 weeks of treatment:						
Left	655	736	694	716	460	667**
Right	518	664*	609	640	420	579*
After 4 weeks of recovery:						
Left	722	729	803	783	484	550
Right	608	654	650	677	438	495**
After 13 weeks of recovery:						
Left	749	762	803	806	610	625
Right	668	680	679	694	477	536**
Basophilia of parotid acinar cells						
After 4 weeks of treatment:						
Minimal	0	1	1	4	6	0
Slight	0	6	0	1	1	0
Moderate	0	1	0	2	0	0
Marked	0	0	0	0	0	8
After 4 weeks of recovery:						
Minimal	1	1	0	0	0	5
Slight	0	0	0	0	0	1
After 13 weeks of recovery:						
Minimal	1	1	1	1	1	2
Slight	0	0	0	0	0	2
Moderate	0	0	0	0	0	1

From Allen (1996)
AP, Alpk:ApfSD (Wistar-derived) rats; CD, Sprague-Dawley (Charles River CD); F344, Fischer 344 rats (F344)
$p > 0.05$, ** $p < 0.01$, Student's t-test, two-sided
[a] Organ weight adjusted for body weight

changes were not completely reversible after 13 weeks of recovery, while in AP and CD rats complete improvement was apparent after a 4-week recovery period (Allen, 1996).

The hypothesis that glyphosate produced the salivary gland changes via β-adrenergic activity was questioned in a recent review paper (Williams et al., 2000). The authors emphasized that, first, if glyphosate was a β-agonist, its effect would be to stimulate β-receptors in other effector organs and produce a characteristic set of cardiocirculatory effects, such as increased heart rate and cardiac output as well as decreased blood pressure and peripheral resistance. None of these effects were noted in other studies. Similarly, it is known that isoproterenol and other β-agonists cause myocardial necrosis and enlargement of heart ventricles after prolonged treatment. Glyphosate did not produce any effects in heart tissue, even after long-term exposure at very high doses, providing additional support for the argument that glyphosate does not act as a β-agonist. The authors concluded that glyphosate has no significant β-adrenergic activity and therefore could not produce salivary gland changes via β-agonist activity. They proposed a number of other potential mechanisms for salivary gland alteration, including non-chemical modes of action. For example, salivary gland secretion has been shown to be affected by the texture and moistness of feed, and salivary gland enlargement has been caused by malnutrition. Glyphosate could be acting by such a non-chemical mechanism. Because glyphosate is a strong organic acid, dietary administra-

tion at relatively high concentrations may cause mild oral irritation leading to increased salivary gland size and flow. In the long-term exposure studies with glyphosate there were several salivary gland changes. These changes were: most pronounced in the parotid gland, responsible for secretion of serous fluid in response to such stimuli as acidic materials; absent in the sublingual gland that releases mucous fluid in response to other stimuli; and observed to an intermediate degree in the submandibular gland that contains a mixture of mucous and serous secreting cells. This pattern of observations was considered to be consistent with the hypothesis that the salivary gland changes observed are a biological response to the acidic nature of glyphosate. These salivary gland alterations are not known to represent any pathological condition and were not considered to be either toxicologically significant or adverse by Williams et al. (2000).

(c) Potential for endocrine modulation

In short-term studies of toxicity performed by the NTP, glyphosate (purity, 99%) was administered to groups of 10 male and 10 female $B6C3F_1$ mice and to groups of 10 male and 10 female F344/N rats at dietary concentrations of 0, 3125, 6250, 12 500, 25 000 or 50 000 ppm for 13 weeks. Evaluations of reproductive tissue revealed a significant reduction (80% of values for controls) of caudal epididymal sperm concentrations in male rats at the two highest doses; however, all values were within the normal range for the historical controls for this strain. All other parameters examined (left caudal, epididymal and testicular weights, epididymal sperm motility, total spermatid heads/testes, and total spermatid heads/gram caudal tissue) were not different from controls in rats or mice. The length of the estrus cycle was slightly longer (5.4 days compared with 4.9 days) in female rats at the highest dose than in the controls, but the biological significance of these findings, if any, is not known (Chan & Mahler, 1992).

In a non-guideline in-vitro experiment, glyphosate and 48 other chemicals were tested in two complementary assays, one measuring activation of the estrogen receptor of the rainbow trout in a yeast system and the other evaluating vitellogenin production in a trout liver cell-culture system. Glyphosate had no estrogenic activity in either assay (Petit et al., 1997).

In a non-guideline in-vitro experiment, glyphosate and eight pesticide formulations were tested for their ability to inhibit steroidogenesis in mouse MA-10 Leydig tumour cells. While glyphosate did not alter steroidogenesis (progesterone production) or total protein synthesis at any dose tested (0–100 µg/ml), the glyphosate formulation Roundup decreased progesterone production in a dosage-dependant manner without a parallel decrease in total protein synthesis (Walsh et al., 2000).

(d) Studies on the metabolite aminomethylphosphonic acid (AMPA)

(i) Acute toxicity

In a study of acute oral toxicity that was performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 401), five male and five female Alpk:AP_fSD (Wistar-derived) rats received AMPA (purity, 100%; in 0.5% aqueous polysorbate 80) as a single dose at 5000 mg/kg bw by gavage. A standard volume of 10 ml/kg bw was given to each animal. Test substance application was followed by a 15-day post-observation period before all the animals were killed and subjected to a macroscopic examination post mortem. None of the rats died before scheduled termination.

Signs of slight toxicity were seen in all animals, but these did not persist and all animals had recovered by day 4. Initially, all animals lost weight, but had exceeded their initial weight by day 6. However, body-weight reduction was noted in two males and three females between days 8 and 15. No treatment related findings were seen at examination post mortem. Accordingly, the acute oral LD_{50} for AMPA was >5000 mg/kg bw in male and female rats (Leah, 1988).

In a study of acute oral toxicity that was performed in compliance with the principles of GLP and according to the test guidelines of the US EPA, five male and five female Sprague-Dawley rats received AMPA (purity, 99.2%; dissolved in 0.5% carboxymethylcellulose) as a single dose at 5000 mg/kg bw by gavage (dose volume, 10 ml/kg bw). The animals were observed frequently on the day of dosing and then once daily over the 14-day observation period. They were weighed before dosing, 7 days after dosing and at sacrifice on day 14. All rats were subjected to a gross examination post mortem. There were no treatment-related mortalities. Clinical signs were observed 4 h after dosing and included piloerection, diarrhoea, subdued behaviour, hunched appearance, and soiled anal and perigenital areas. All animals recorded normal body-weight gain throughout the experiment. No abnormalities were detected at necropsy after 14 days observation. Thus, the acute oral LD_{50} of AMPA in rats is >5000 mg/kg bw (Cuthbert & Jackson, 1993a).

In a study of acute dermal toxicity performed in compliance with the principles of GLP and according to the test guidelines of the US EPA, five male and five female Sprague-Dawley rats received AMPA (purity, 99.2%) as a single dose at 2000 mg/kg bw. The test substance was administered evenly onto a square dressing (5 cm × 5 cm) that was moistened with distilled water and then applied to the shaved back of each rat. The patch was covered with an occlusive dressing and kept in contact with the skin for 24 h. At the end of the exposure period the patch was removed and the exposed skin wiped with distilled water to remove any excess test material. The rats were observed frequently on the day of dosing and then once a day over the 14-day observation period. At study termination, animals were sacrificed and subjected to necropsy. There were no mortalities after a single dermal application of AMPA at 2000 mg/kg bw, no clinical signs were noted and no abnormalities detected at necropsy. Thus, the acute dermal LD_{50} of AMPA to rats must be above this limit dose (Cuthbert & Jackson, 1993b).

The dermal sensitization potential of AMPA (purity, 99.2%) was evaluated in a Magnusson & Kligman maximization test performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 406). On the basis of the results of a preliminary test, a group of 20 female Dunkin-Hartley guinea-pigs received AMPA by an intradermal injection (10% w/v in carboxymethylcellulose) and 6 days later by topical application (25% w/v in carboxymethylcellulose). Slight to moderate skin irritation was observed at the treated sites. Two weeks after the topical induction, the animals were challenged with AMPA (25% w/v in carboxymethylcellulose). The skin reactions were scored 24 h and 48 h after removal of the patches. None of the animals showed a positive response at challenge (Cuthbert & Jackson, 1993c).

(ii) Short-term studies of toxicity

In a range-finding study performed in compliance with the principles of GLP, groups of five male and five female Sprague-Dawley rats were given AMPA (purity, 99.2%; in carboxymethylcellulose) at doses of 0, 10, 100, 350 or 1000 mg/kg bw per day by oral gavage for 28 days. Control animals were given carboxymethylcellulose alone (at a volume of

10 ml/kg bw). Animals were observed daily for mortalities and signs of reaction to treatment. Once per week all animals received a detailed clinical examination. Body weights and food consumption were calculated weekly, water consumption was monitored by visual inspection throughout the study. At study termination all animals were sacrificed and necropsied. Thirteen different tissues were weighed and fixed for histological examination.

There were no mortalities or clinical signs observed throughout the duration of the study. There were no notable intergroup differences with regards to body weight in males. Females receiving a dose of 1000 mg/kg bw per day displayed a slight reduction by 13% in body weight when compared with values for the controls. However, this change was not statistically significant. In males, a similar effect was not observed. There were no notable intergroup differences in food and water consumption for males and females. Furthermore, there were slight but statistically significant increases in kidney weights in males at the two higher doses when compared with values for the control group (by 7% and 8%, respectively). Histological examinations revealed a very mild reduction of serous secretion in the mandibular salivary gland of males at the highest dose. With regard to the salivary gland findings in some of the studies with glyphosate, it is equivocal whether or not this minor finding was related to treatment.

On the basis of an increase in kidney weight in male rats at 350 and 1000 mg/kg bw per day and a reduction of body weight in females at the highest dose, the NOAEL for AMPA was 100 mg/kg bw per day (Heath et al., 1993).

In a study performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 408), groups of 10 male and 10 female Sprague-Dawley rats were given AMPA (purity, 99.2%; in carboxymethylcellulose) at a dose of 0, 10, 100 or 1000 mg/kg bw per day by oral gavage for 13 weeks. Animals in the control group were given carboxymethylcellulose alone (at a volume of 10 ml/kg bw). Animals were observed daily for mortalities and signs of reaction to treatment. Once per week all animals received a detailed clinical examination. Body weights and food consumption were recorded weekly; water consumption was monitored by visual inspection throughout the study. Ophthalmoscopy examinations were performed on all animals during pretrial and on all animals in the control group and at the highest dose during week 12 of dosing. Blood samples were collected from the orbital sinus of all animals during week 13 of dosing. The blood collected was analysed for 14 haematology and 14 clinical chemistry parameters. At study termination all animals were sacrificed and necropsied. Fourteen organs including submaxillary, sublingual and parotid salivary glands were removed and weighed. Fourty tissues from premature decedents and from animals in the control group and the group receiving the highest dose were collected and fixed for full histopathological examination.

There were no unscheduled deaths that could be attributed to treatment and no specific clinical signs were noted over the course of the study. Ophthalmoscopy examinations resulted in no abnormal findings. There were no dose-related intergroup differences with regard to body weight, food or water consumption in any sex throughout the study. Haematology and clinical chemistry revealed a few minor effects, however, in the absence of a clear dose–response relationship, these were considered to have occurred by chance. There were no significant organ weight changes attributed to treatment with AMPA. The effect on kidney weight as elucidated in the previous 4-week study was not confirmed. At necropsy, none of the findings could be attributed to administration of AMPA. The NOAEL in this study was 1000 mg/kg bw per day, i.e. the highest dose tested (Strutt et al., 1993).

(iii) Genotoxicity

In an Ames test performed under GLP conditions and in compliance with OECD 471, AMPA (purity, >99% w/w) was tested in the presence and absence of metabolic activation (S9 mix) in two independent tests using five strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98 and TA100) and one strain of *Escherichia coli* (WP2*uvrA* pKM101). AMPA was dissolved in sterile deionized water and tested at doses of between 1.6 and 5000 µg/plate. The incubation period was 72 h at 37 °C. Appropriate controls were included. The number of revertant colonies was determined using an automated electronic colony counter.

In two separate assays, the test substance did not induce any significant, reproducible increases in the observed number of revertant colonies in *S. typhimurium* strains TA1535, TA1538, TA98 and TA100 or *E. coli* strain WP2*uvrA* pKM101 either in the presence or absence of an auxiliary metabolizing system (S9 mix). In contrast, the positive control substance caused an increase in the mean number of revertant colonies, thus demonstrating the sensitivity of the test system to a known mutagen. In the first test, small and non-dose related increases in revertant colony numbers were observed with strain TA1537 both in the presence and absence of metabolic activation. Increases were not seen in two further independent tests with this strain. This lack of reproducibility proved that the originally observed increases were not indicative of mutagenic activity and that AMPA uniformly gave a negative, i.e. non-mutagenic, response under the conditions of this assay (Callander, 1988).

In an Ames test performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 471), cultures of four mutant strains of *S. typhimurium* (TA100, TA98, TA1537 and TA1535) were exposed to AMPA (purity, 99.2%) at a concentration of 5.0, 2.5, 1.3, 0.63 and 0.31 mg per plate with and without metabolic activation (S9 mix). Two independent test series were performed, the first as a plate incorporation assay, the second as a preincubation assay using replicates of three plates for each dose. Positive and negative controls were included in both tests. After incubation for 48–72 h at 37 °C, the number of colonies (revertants) were counted.

The counts for negative and positive controls were all within the expected ranges. No depression of background growth was observed, indicating that AMPA was not cytotoxic at concentrations of up to 5.0 mg/plate. The number of revertants groups treated with glyphosate were generally similar to those in the concurrent controls. A single statistically significant increase in revertants was found (TA1535, plate incorporation assay, at a concentration of 0.63 mg/plate without metabolic activation). However, the increase was marginal and no dose–response relationship was seen. Thus, AMPA was found to be non-mutagenic in this test system (Jensen, 1993a).

In a test for mammalian cell gene mutation in vitro, performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 476), AMPA (purity, 99.2%) at a dose of 0.31, 0.63, 1.3, 2.5 or 5.0 mg/ml was applied to cell cultures of mouse lymphoma cells (L5178Y), with and without metabolic activation. Duplicate tests were performed for each dose. Solvent controls and positive controls were included in both assays. After incubation for 10 days at 37 °C, the numbers of cell clones were counted and the cloning efficiencies determined.

The mutation frequencies of the test cultures were generally similar to those of the concurrent negative controls at all doses, with and without metabolic activation. Statistical

analysis revealed no statistically significant differences at any dose. The mutation frequency for positive controls was in the expected range in all test series. Thus, AMPA was found to be non-mutagenic in L5178Y mouse lymphoma cells in vitro (Jensen, 1993b).

In an assay for micronucleus formation, performed in compliance with the principles of GLP and according to the test guidelines of the US EPA and the OECD (TG 474), groups of five male and five female Bom:NMRI mice received AMPA (purity, 99.2%) as a single dose at 5000 mg/kg bw by oral gavage. The test substance was dissolved in aqueous sodium chloride and carboxymethylcellulose to give a dose volume of 20 ml/kg bw. Positive and solvent control groups of similar size were also included. The mice from the different AMPA test groups were killed at 24, 48 and 72 h, respectively, after dosing. The negative (solvent) control group mice were all sacrificed after 48 h and the positive control group terminated 24 h after dosing. Immediately after a mouse was killed, cell smears were prepared. Slides were coded in order to perform a blind counting. The following counts were made: percentage of polychromatic erythrocytes (PCE) in 2000 erythrocytes—% PCE; number of micronuclei (MN) observed in 2000 polychromatic erythrocytes (PCE)—MN/PCE; and the number of micronuclei in normochromatic erythrocytes (NCE) observed during the counting of 2000 PCE—MN/NCE.

The % PCE in the AMPA test groups was significantly lower than that in the control group, indicating a clear depression of erythropoiesis. The frequency of micronucleus formation in the positive and negative control groups were in accordance with historical data. The frequency of micronucleus formation in PCE was similar in the negative control and test groups. The results for AMPA were negative in this assay for micronucleus formation in vivo (Jensen, 1993c).

(iv) Developmental toxicity

In a study of devlopmental toxicity performed in compliance with the principles of GLP and roughly according to the test guidelines of the US EPA and the OECD (TG 414), groups of 10 mated female Sprague-Dawley rats received AMPA (purity, 99.2%; in carboxymethylcellulose) at a dose of 100, 350 or 1000 mg/kg bw by oral gavage from day 6 to day 16 of gestation. Control animals were dosed with carboxymethylcellulose and distilled water alone. Animals were observed daily for mortalities and reaction to treatment. Individual body weights were recorded on days 0, 6, 9, 13, 17 and 20 of gestation. Food consumption was recorded daily, starting on day 4 of gestation. On day 20 of gestation, animals were sacrificed to examine congenital abnormalities and macroscopic pathological changes in maternal organs. The ovaries and uteri were examined to determine the number of corpora lutea and number and position of all implantation sites in the uterus. Each implant was classified as being: (a) live; (b) a fetal death, judged to have occurred after day 16 of gestation; (c) a late embryonic death, judged to have occurred in the period between day 12–16 of gestation; or an early embryonic death, judged to have occurred before day 12 of gestation. Live fetuses were individually identified, weighed and examined for any externally visible abnormalities. Half the fetuses were examined for visceral and skeletal abnormalities and the remaining fetuses were examined for soft tissue abnormalities.

There were no mortalities, and there were no clinical observations related to treatment with AMPA throughout the duration of the study. Body-weight gain and food consumption of the test animals were similar to those of the controls. There were no notable intergroup differences in the incidence of intrauterine deaths, or in mean fetal weights. Examination

of fetuses for developmental abnormalities and variations of the viscera and skeleton (including state of ossification) showed no intergroup differences. Thus, the maternal and developmental NOAEL was 1000 mg/kg bw per day, the highest dose. No evidence of teratogenicity was obtained (Hazelden, 1992).

3. Observations in humans

There are various reports in the literature describing the effects observed after accidental and/or intentional ingestion of concentrated formulations of glyphosate. Large amounts of glyphosate-based herbicides are occasionally deliberately ingested to attempt suicide, mainly in Asian countries, and may result in serious gastrointestinal, cardiovascular, pulmonary and renal effects and possibly death (Talbot et al., 1991; Tominack et al., 1991; Lee et al., 2000). The nature of the clinical symptoms suggests that hypovolemic shock was the cause of death (Sawada et al., 1988; Tominack et al., 1989). It has been pointed out by these authors that the surfactant contained in glyphosate formulations may be responsible for the clinical syndrome, but that the available evidence on this point was inconclusive. In such cases, aggressive supportive care is recommended (Tominack et al., 1989). Accidental exposure to small volumes of glyphosate results in, at most, only mild effects; no deaths have been reported (Goldstein et al., 2002).

In spite of this experience, it has been stated that glyphosate is a leading cause of pesticide poisoning. This contradiction may be elucidated using data from California. The claims are based upon a counting of telephone calls to the California Environmental Protection Agency's Pesticide Poisoning Information System (PISP). Since the inception of the PISP database, glyphosate has been among the most frequently reported individual agents (California EPA, 1996). Review of the California data indicates that the number of reported cases simply reflects greater use of the product relative to other herbicides and shows that glyphosate has relatively low toxicity among pesticides used in California (Goldstein et al., 2002). PISP was created in 1982 as a clearinghouse for telephone calls of pesticide-related illness. Concurrently, the reporting of pesticide-related illness to PISP was made mandatory for health-care providers in California. The data collected there include cases of eye and skin irritation, systemic symptoms, as well as general inquiries and asymptomatic exposures. Thus, number of calls is a poor indicator of true "clinical poisoning", defined as a poisoning with the occurrence of systemic symptoms and excluding those cases involving only topical irritation of the skin and/or eye. An analysis of the database spanning 1982 to 1997 shows that there were 815 calls involving glyphosate herbicide products. Of those 815 calls, 399 were eye irritation-only cases, 250 were skin irritation-only cases, seven were respiratory-only cases and 32 were mixed cases (eye, skin and respiratory). Only 20 out of the 815 calls reported systemic symptoms after use of a glyphosate product only. The reported symptoms were not severe, expected to be limited in duration, and were frequently inconsistent with the route of exposure and/or previous experience with glyphosate.

The California Department of Pesticide Regulation noted in its 1994 report that most people (>80%) affected by glyphosate experienced only irritant effects and, of the 515 pesticide-related hospitalizations recorded over 13 years, none was attributed to glyphosate.

Acquavella et al. (1999) evaluated ocular effects in 1513 cases of exposure to glyphosate formulations reported to a certified regional centre of the American Association of Poison Control Centres from 1993 to 1997. The large majority of reported exposures were judged by specialists at the centre to result in either no injury (21%) or only transient

minor symptoms (70%). None of the reported exposures resulted in permanent change to the structure or function of the eye. This information is particularly important since glyphosate acid was irritant to the eyes in studies in animals.

Barbosa et al. (2001) published a single case report of a man aged 54 years who accidentally sprayed himself with a glyphosate-based formulation in his garden (manufacturer and formulation details unknown). According to the authors, within 6 h of the incident the man developed conjunctival hyperaemia and a generalized rash. One month later he presented with Parkinsonian symptoms in all four extremities and 1 year later developed a resting tremor of one hand and complained of memory deficits. However, this single case is not sufficient to prove the proposed relationship between exposure to glyphosate and the occurrence of Parkinson disease, since this finding is inconsistent with extensive testing in animals, and human experience. Furthermore, the hypothesis regarding a possible mechanism of action via production of glycine is not supported by existing metabolic data. There is no credible evidence so far that glyphosate is capable of inducing Parkinson disease or any other neurological illness in humans or animals.

Exposure related to the professional use of glyphosate-based formulations, through the monitoring of the single active ingredient glyphosate, has been the subject of a number of studies. The practices monitored in those studies represent a range of application techniques, use rates, workloads and reflect variety in use of personal protective equipment. Dermal contact is the most likely route of exposure for applicators; and activities such as mixing and loading of glyphosate and extended applications using hand sprayers have the highest potential for exposure. Inhalation is considered to be a minimal route of exposure under most circumstances because of glyphosate's extremely low vapour pressure.

Both passive dosimetry and biomonitoring have been used as techniques to assess exposure. Biomonitoring results represent systemic (internal) exposure, while the results obtained from passive dosimetry quantify external deposition. There is general agreement that biological measurements as obtained through biomonitoring provide the most relevant information for safety assessments (Chester & Hart, 1986; Franklin et al., 1986). Biomonitoring for glyphosate has been a particularly valuable technique because metabolism studies have shown that it is rapidly excreted by mammals unchanged, primarily via urine, facilitating interpretation of exposure with little need for adjustment of the results to account for pharmacokinetic factors.

Some biomonitoring studies were performed on silvicultural workers who sprayed a glyphosate formulation in a variety of forestry and tree farming activities. In one study, the United States Department of Agriculture's Forest Service, in collaboration with Monsanto Company and the University of Arkansas, sponsored a study to investigate exposure of workers to glyphosate at two forestry nurseries in Oregon and in Massachusetts where glyphosate was used for weed control (Lavy et al., 1992). At both nurseries, exposure of applicators, weeders, and scouts were measured while they performed their normal duties. They assessed the internal dose of glyphosate through analyses of the total daily urine excreted by each of the workers. Urine samples were collected from the weeders and scouts before working with glyphosate and for 8 months thereafter. Continuous sampling of total urine was conducted for the first 12 consecutive weeks of the study, after which a 24-h sample was collected each Wednesday for the next 5 months. Urine samples from applicators were collected for a 6-day period, including the day before, the day of, and the 4 days after the applications of herbicide. These samples were analysed as 24-h composites. Of the

355 daily samples of urine analysed, none were found to contain quantifiable concentrations of glyphosate. The limit of quantification was 10 ppb. Dermal exposure was likely for the workers; the lack of quantifiable glyphosate in the urine was attributed to the very limited ability of glyphosate to penetrate the skin of the exposed workers.

In a second collaborative study conducted by the US Department of Agriculture Forestry Service, Georgia Tech Research Institute, and Monsanto (Cowell & Steinmetz, 1990), the exposure of applicators to glyphosate during a hand-held directed spray foliar application at three sites maintained by the Forestry Service where glyphosate was used to control vegetative growth around pine seedlings planted in clear-cut forest areas was assessed. At each test site, in addition to applying the herbicide, one person (the mixer) measured and mixed a 3% (v/v) spray solution of the formulation and filled the backpack sprayers for all the other applicators. At all three sites, five workers per site applied glyphosate, and were monitored for exposure to glyphosate on the day the applications were made. In addition, at one site a supervisor also applied glyphosate and was monitored for exposure. Urine samples for biological monitoring from each participant were collected for a period of 5 days. Urine was collected the day before, the day of, and the 3 days after the application of glyphosate. Urine specimens for each worker were combined to form 12-h composite samples. Of the 96 urine samples analysed, five were found to contain quantifiable levels of glyphosate. The highest concentration of glyphosate measured was 14 ppb and the highest estimated internal dose was 0.0006 mg/kg bw, which is well below the proposed ADI.

Two other studies have been conducted to measure exposure of forestry workers to glyphosate during normal silvicultural applications: one in Finland (Jauhiainen et al., 1991) and the other in Canada (Center de Toxicologie du Quebec, 1988).

For the Finnish study, two groups of five forestry workers were used: an unexposed control group of workers that planted young trees, and a test group of workers that applied glyphosate using brush saws equipped with pressurized sprayers. The test group sprayed glyphosate each day for 5 consecutive days in August 1988. Each worker prepared fresh spray solutions each day. Urine samples were taken at the end of each working day that glyphosate was applied. Urine samples were also taken from each of the workers 3 weeks after the last day of herbicide application. In addition, each worker received a medical examination on the first and last days that glyphosate was applied and a follow-up examination 3 weeks after the last application day. These examinations included haematology, clinical chemistry, electrocardiogram (ECG), pulmonary function tests, an interview for a health questionnaire, and a general clinical examination (including blood pressure, pulse rate and pressure craft of hands). All urine samples had less than detectable concentrations of glyphosate. There were no statistically significant differences in the findings of the medical examinations conducted before and after exposure.

The Canadian study of exposure of forestry workers to glyphosate after normal silviculture uses of glyphosate was conducted over two growing seasons and involved 45 workers. During the summer of 1986 a crew of five forestry workers (foreman, mixer, operator, and two flagmen) in charge of spraying operations were monitored for exposure to glyphosate. Seven urine samples were collected from each worker on each day of herbicide application: one at the beginning of the work day, four during the course of the work day, one at the end of the work day, and one in the morning of the day after application. Glyphosate applications were made on 19 different days, with the total application times

ranging from 1 to 9 h per day. The active ingredient was not detected in most urine samples from the two flagmen and the operator, and concentrations of glyphosate in all urine samples were <0.03 ppm (the limit of quantitation). In contrast, 14 out of 33 urine samples from the mixer and two urine samples for the foreman contained glyphosate at concentrations of >0.03 ppm. Maximum glyphosate concentrations in the foreman's and mixer's urine were 0.043 and 0.055 ppm, respectively.

As a follow-up to the 1986 study, 40 forestry workers were monitored during the summer of 1987 for exposure to glyphosate during normal use of glyphosate in silviculture. Consistent with the results of previous studies, concentrations of glyphosate in the urine of exposed workers were very low. In most samples, glyphosate was not detectable. In those samples that did contain detectable levels of glyphosate, concentrations were <0.1 ppm in all cases, and typically <0.035 ppm.

Although the concentrations of glyphosate in some of the urine samples of workers in this study were greater than those found in other glyphosate worker exposure studies, the levels found were very low.

The most recent biomonitoring study, the Farm Family Exposure Study (FFES), was funded through a research contract with the University of Minnesota and sponsored by seven agricultural chemical companies in order to investigate real-world exposures to pesticides for farmers and their families using state-of-the-art field and analytic methods. FFES participants were randomly selected from licensed pesticide applicators in Minnesota and South Carolina. Families were eligible if there was a farmer, spouse, and at least one child aged 4–18 years living on the farm; if they owned or leased at least 10 acres (0.04 km^2) of cropland; if they planned to apply at least one of the target pesticides (glyphosate, 2,4-D, or chlorpyrifos) within 1 mile (1.6 km) of their residence; if they were willing to collect all their urine for 5 consecutive days (the day before, the day of, and the 3 days after a pesticide application); and if they were willing to fill out pre-application and post-application questionnaires. FFES field staff observed all pesticide applications and documented information relevant for exposure assessment. Forty-eight farm families, including 79 children, provided urine specimens relating to glyphosate application. Analysis of 24-h composite urine samples was performed for each family member the day before, the day of, and for 3 days after a glyphosate application. The limit of detection (LOD) was 1 ppb.

Twenty-nine out of 48 farmers (60%) were found to have detectable levels of glyphosate in their urine on the day of application. The geometric mean concentration was 3.2 ppb on the day of application and declined thereafter, and the maximum concentration was 233 ppb. Farmers who did use rubber gloves when mixing and loading glyphosate formulations had lower geometric mean urinary concentrations than those who did not (2.0 ppb compared with 9.7 ppb). The number of acres treated was not correlated with urinary concentration of glyphosate, but there was a trend between concentration and the number of times that farmers mixed and loaded the concentrated herbicide formulation. Other factors associated with urinary concentration of glyphosate were using an open cab tractor, observed skin contact with the glyphosate formulation, and repairing equipment during the application.

Detectable urinary concentrations of glyphosate were infrequent for farm spouses and farm children. Two out of 48 farm spouses (4%) had detectable values on the day of application, the highest individual concentration was 3 ppb. Of the 78 children who provided samples on the day of application, nine (12%) had a detectable glyphosate concen-

tration, and all except one of the children had been present for or assisted with mixing, loading, or application activities. The maximum urinary concentration of glyphosate, 29 ppb, was for a teenage boy who assisted his father with the mixing and application. The maximum systemic dose of glyphosate for farmers, spouses, and children was estimated to be 0.004, 0.00004 and 0.0008 mg/kg bw, respectively (Acquavella et al., 2004).

Widely used pesticides, like glyphosate, have recently become a focus of epidemiological research. In the past few years several epidemiological studies have been published that reported weak associations of glyphosate with lymphopoeitic cancers (Nordstrom et al., 1998; Hardell & Erikson, 1999; McDuffie et al., 2001), self-reported adverse reproductive outcomes (Savitz et al., 1997; Curtis et al., 1999; Arbuckle et al., 2002) and self-reported attention deficit hyperactivity disorder in children (Garry et al., 2002). However, the results of these studies do not meet generally accepted criteria from the epidemiology literature for determining causal relationships. Generally, the associations were rather weak and rarely statistically significant. Control for potential confounding factors, including other pesticides, was not possible owing to limited available information and small numbers of subjects. It was not measured whether there actually was any internal exposure or the extent of such exposure and, accordingly, a possible dose–response relationship could not be evaluated.

Comments

After oral administration to rats, [^{14}C]glyphosate was only partially absorbed (about 30–36%) from the gastrointestinal tract. Absorption was not significantly dose-dependent over the range of 10 to 1000 mg/kg bw. Peak plasma concentrations of radiolabel were observed at 0.5–1 h after dosing in rats and hens, respectively, and at 6–8 h after dosing in goats. The highest tissue concentrations were found in bone, with lower concentrations being found in bone marrow, kidney and liver. After oral administration, about 60–70% of the administered dose was eliminated in the faeces. Of the glyphosate that was absorbed, most was excreted in the urine and <0.2% in expired air. After intravenous application, faecal excretion via bile was only about 2–8% of the administered dose. Whole-body clearance (about 99% of an oral dose) occurred within approximately 168 h. The estimated half-life for whole-body elimination of the radiolabel was 2.1–7.5 h for the alpha phase and 69–337 h for the beta phase. Repeated dosing did not alter absorption, distribution, and excretion. There was very little biotransformation of glyphosate; the only metabolite, AMPA, accounted for ≤0.7% of the administered dose in excreta; the rest was unchanged glyphosate.

Glyphosate has low acute oral toxicity in mice (LD_{50}, >2000 mg/kg bw; no deaths at this dose) and rats (LD_{50}, >5000,mg/kg bw), low acute dermal toxicity in rats (LD_{50}, >2000 mg/kg bw) and rabbits (LD_{50}, >5000 mg/kg bw), and low acute inhalation toxicity in rats (LC_{50}, >4.43 mg/l). Clinical signs after acute oral exposure included reduced activity, ataxia and convulsions.

Glyphosate was not irritating to the skin, but produced moderate to severe eye irritation with irreversible corneal opacity in one study. Glyphosate salts were slightly irritating to the eye, with minimal to moderate conjunctival irritation and slight iritis that usually disappeared within 48 h after exposure. Glyphosate was not a skin sensitizer in guinea-pigs.

In short-term studies of toxicity in different species, the most important effects were clinical signs related to gastrointestinal irritation, salivary gland changes (hypertrophy and increase in basophilia of cytoplasm of acinar cells) and hepatotoxicity. In mice, reduced body-weight gain was seen at a dietary concentration of 25 000 ppm. Alterations of the salivary glands were present in mice in one of two short-term studies at dietary concentrations of ≥6250 ppm; the NOAEL for this finding was 3125 ppm (equal to 507 mg/kg bw per day). In rats, findings included soft faeces, diarrhoea, reduced body-weight gain, decreased food utilization and slightly increased plasma enzyme activities (ALP, ALT) at dietary concentrations of ≥20 000 ppm. Additionally, in two out of four 90-day studies in rats, increased incidences of alterations of the salivary glands were observed. At the lower doses, these changes were only minimal with respect to severity and incidence. The overall NOAEL was 300 mg/kg bw per day.

In dogs, the NOAEL in a 90-day feeding study was 10 000 ppm (equal to 323 mg/kg bw per day) on the basis of reduced body-weight gain, marginal reductions in albumin and calcium concentrations, and increased plasma ALP activities at 50 000 ppm. In a 1-year study in dogs given capsules containing glyphosate, the NOAEL was 30 mg/kg bw per day, on the basis of clinical signs (soft faeces, diarrhoea) and reduced body-weight gain at ≥300 mg/kg bw per day. In a 1-year feeding study, the NOAEL was 15 000 ppm (equal to 440 mg/kg bw per day) on the basis of reduced body-weight gain at 30 000 ppm.

Long-term studies of toxicity and carcinogenicity were conducted in mice and rats. In the study of carcinogenicity in mice, no toxic effects were observed at up to the highest dose tested (1000 mg/kg bw per day), and there was no evidence of carcinogenicity.

In a 1-year study of toxicity in rats, the NOAEL was 2000 ppm (equal to 141 mg/kg bw per day) on the basis of a reduction in body weight and clinical chemistry findings at 8000 ppm. Three new long-term studies in rats were evaluated. In the first study, the NOAEL was 8000 ppm (equal to 362 mg/kg bw per day) on the basis of a reduction in body weight in females and an increased incidence of cataracts and lens abnormalities in males at 20 000 ppm. In the second study, the NOAEL was 100 mg/kg bw per day on the basis of more pronounced alterations of the parotid and submaxillary salivary glands at ≥300 mg/kg bw per day. In the most recent 2-year study in rats, the NOAEL was 6000 ppm (equal to 361 mg/kg bw per day) on the basis of a reduction in body weight and food consumption, and indications of kidney, prostate, and liver toxicity at 20 000 ppm. There was no evidence of a carcinogenic response to treatment in rats.

The genotoxic potential of glyphosate has been extensively tested in a wide range of assays both in vitro and in vivo, including end-points for gene mutation, chromosomal damage and DNA repair. Negative results were obtained in studies performed in compliance with current test guidelines. The Meeting concluded that glyphosate is unlikely to be genotoxic.

In view of the absence of a carcinogenic potential in animals and the lack of genotoxicity in standard tests, the Meeting concluded that glyphosate is unlikely to pose a carcinogenic risk to humans.

Glyphosate had no effects on fertility in both two-generation studies of reproductive toxicity in rats. The overall NOAEL for parental and offspring toxicity was 3000 ppm (equal to 197 mg/kg bw per day) on the basis of increased food and water consumption and reduced

body-weight gain in F_1 animals, and an increased incidence of alterations of the parotid and submaxillary salivary glands in F_0 and F_1 animals at 10 000 ppm.

In studies of developmental toxicity in rats, the NOAEL for maternal and developmental toxicity was 300 mg/kg bw per day, on the basis of clinical signs and reduced body-weight gain in the dams and increased incidences of fetuses with delayed ossification and skeletal anomalies.

In studies of developmental toxicity in rabbits, the NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of clinical signs and reduced food consumption and body-weight gain. The NOAEL for developmental toxicity was 175 mg/kg bw per day on the basis of reduced fetal weight and delayed ossification, and an increased incidence of postimplantation loss. The Meeting concluded that glyphosate is not teratogenic.

The Meeting concluded that the existing database on glyphosate was adequate to characterize the potential hazards to fetuses, infants, and children.

Hypertrophy and cytoplasmic alterations of the salivary glands (parotid and/or mandibular) was a common and sensitive end-point in six studies: in three 90-day studies (one in mice, two in rats), a 1-year study in rats, a 2-year study in rats and a two-generation study of reproductive toxicity in rats. Mechanistic studies available to the Meeting hypothesized that the mechanism was adrenergic. However, the inability of a β-blocker to significantly inhibit these effects indicates that glyphosate does not act as a β-agonist. Other proposed mechanisms for the salivary gland alterations include oral irritation caused by dietary administration of glyphosate, a strong organic acid. Although the mechanism of the cytoplasmic alterations in the salivary glands was unclear, the Meeting concluded that this treatment-related effect is of unknown toxicological significance.

In a study of acute neurotoxicity in rats, the NOAEL for neurotoxicity was 2000 mg/kg bw, the highest dose tested. In a short-term study of neurotoxicity in rats, the NOAEL for neurotoxicity was 20 000 ppm, equal to 1547 mg/kg bw per day, the highest dose tested. In a study of acute delayed peripheral neuropathy in hens, clinical and histopathological examination found no evidence for acute delayed peripheral neuropathy at a dose of 2000 mg/kg bw.

New toxicological data on AMPA (the primary degradation product of glyphosate in plants, soil and water, and the only metabolite of glyphosate found in animals) was submitted to the present Joint Meeting for evaluation. AMPA was of low acute oral and dermal toxicity in rats (LD_{50}, >5000 and >2000 mg/kg bw, respectively), and was not a skin sensitizer in guinea pigs. In a 90-day study of toxicity in rats, the NOAEL was 1000 mg/kg bw per day, the highest dose tested. AMPA had no genotoxic potential in vitro or in vivo. In a study of developmental toxicity in rats, no evidence for embryo- or fetotoxicity was found and the NOAEL for maternal and developmental toxicity was 1000 mg/kg bw per day, the highest dose tested.

On the basis of the new toxicological data, the present Joint Meeting concluded that AMPA is of no greater toxicological concern than its parent compound, thus confirming the conclusion of the 1997 JMPR.

Routine medical surveillance of workers in production and formulation plants revealed no adverse health effects attributable to glyphosate. In operators applying

glyphosate products, cases of eye, skin and/or respiratory tract irritation have been reported. Acute intoxication was reported in humans after accidental or intentional ingestion of concentrated glyphosate formulations, resulting in gastrointestinal, cardiovascular, pulmonary, and renal effects and occasionally death. The acute toxicity of glyphosate formulations was likely to be caused by the surfactant in these products.

The Joint Meeting established a group ADI for glyphosate and AMPA of 0–1.0 mg/kg bw on the basis of the NOAEL of 100 mg/kg bw per day for salivary gland alterations in a long-term study of toxicity and carcinogenicity in rats and a safety factor of 100. The ADI is supported by NOAELs of 141 and 197 mg/kg bw per day from the 1-year study and the two-generation study of reproductive toxicity in rats, respectively.

The Joint Meeting concluded that it was not necessary to establish an ARfD for glyphosate in view of its low acute toxicity, the absence of relevant developmental toxicity in rats and rabbits that could have occurred as a consequence of acute exposure, and the absence of any other toxicological effect that would be elicited by a single dose.

The NOAEL of 30 mg/kg bw per day in a 1-year study in dogs was not considered to be relevant for establishing either the ADI or ARfD, since the gastrointestinal effects seen in this study at 300 and 1000 mg/kg bw per day were related to high local concentrations of test substance resulting from the administration of glyphosate in capsules.

Toxicological evaluation

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	3-month study of toxicity[a,e]	Toxicity	3 125 ppm, equal to 507 mg/kg bw per day	6 250 ppm, equal to 1 065 mg/kg bw per day
	2-year study of carcinogenicity[a]	Toxicity	1 000 mg/kg bw per day[d]	—
		Carcinogenicity	1 000 mg/kg bw per day[d]	—
Rat	3-month study of toxicity[a,e]	Toxicity	300 mg/kg bw per day	12 500 ppm, equal to 811 mg/kg bw per day
	1-year study of toxicity[a]	Toxicity	2 000 ppm, equal to 141 mg/kg bw per day	8 000 ppm, equal to 560 mg/kg bw per day
	2-year study of toxicity and carcinogenicity[a,e]	Toxicity	100 mg/kg bw per day	300 mg/kg bw per day
		Carcinogenicity[d]	20 000 ppm, equal to 1 214 mg/kg bw per day[d]	—
	Multigeneration reproductive toxicity[a,e]	Parental toxicity	3 000 ppm, equal to 197 mg/kg bw per day	10 000 ppm, equal to 668 mg/kg bw per day
		Offspring toxicity	3 000 ppm, equal to 197 mg/kg bw per day	10 000 ppm, equal to 668 mg/kg bw per day
	Developmental toxicity[b,e]	Maternal toxicity	300 mg/kg bw per day	1 000 mg/kg bw per day
		Embryo- and fetotoxicity	300 mg/kg bw per day	1 000 mg/kg bw per day
Rabbit	Developmental toxicity[b,e]	Maternal toxicity	100 mg/kg bw per day	150 mg/kg bw per day
		Embryo- and fetotoxicity	175 mg/kg bw per day	300 mg/kg bw per day
Dog	3-month study of toxicity[a]	Toxicity	10 000 ppm, equal to 323 mg/kg bw per day	50 000 ppm, equal to 1 680 mg/kg bw per day
	1-year study of toxicity[a,c,e]	Toxicity	30 mg/kg bw per day[c,f]	300 mg/kg bw per day[c]

[a] Dietary administration
[b] Gavage administration
[c] Capsules
[d] Highest dose tested
[e] Two or more studies combined
[f] Not used for establishing the ADI (or ARfD) since the NOAEL was based on an effect induced by high local concentrations

Estimate of acceptable daily intake for humans

0–1.0 mg/kg bw

Estimate of acute reference dose

Unnecessary

Studies that would provide information useful for continued evaluation of the compound

- Additional information on the mechanism of the changes in the salivary glands
- Further observations in humans

Summary of critical end-points for glyphosate

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of oral absorption	Rapid, approximately 30–36%
Dermal absorption	No information
Distribution	Widely distributed
Rate and extent of excretion	Largely complete within 48 h; approximately 30% in urine and 70% in faeces
Potential for accumulation	No evidence of accumulation (<1% after 7 days)
Metabolism in mammals	Very limited (<0.7%), hydrolysis leading to AMPA
Toxicologically significant compounds (animals, plants and the environment)	Parent compound, AMPA
Acute toxicity	
Rat, LD_{50}, oral	>5000 mg/kg bw
Rat, LD_{50}, dermal	>2000 mg/kg bw
Rat, LC_{50}, inhalation	>4.43 mg/l (4-h, nose-only exposure)
Rabbit, dermal irritation	Non-irritant
Rabbit, eye irritation	Moderately to severely irritant
Skin sensitization	Not sensitizing (Magnusson & Kligman test, Buehler test)
Short-term toxicity	
Target/critical effect	Clinical signs (soft faeces, diarrhoea), reduced body-weight gain; liver (toxicity), salivary glands (hypertrophy)
Lowest relevant oral NOAEL	300 mg/kg bw per day (90-day study in rats)
Lowest relevant dermal NOAEL	—
Lowest relevant inhalation NOAEC	—
Genotoxicity	No genotoxic potential
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Reduced body-weight gain; liver (toxicity), salivary glands (hypertrophy), eye (cataract, lens fibre degeneration)
Lowest relevant NOAEL	100 mg/kg bw per day (2-year study in rats)
Carcinogenicity	No evidence of carcinogenicity in rats or mice
Reproductive toxicity	
Reproductive target/critical effect	Reduced pup weight at parentally toxic doses
Lowest relevant reproductive NOAEL	197 mg/kg bw per day (two-generation study in rats)
Developmental target/critical effect	Embryo- and fetotoxicity at maternally toxic doses (rat, rabbit)
Lowest relevant developmental NOAEL	175 mg/kg bw per day (rabbit)
Neurotoxicity/delayed neurotoxicity	No evidence of neurotoxicty in any study conducted
Medical data	Medical surveillance of workers in plants producing and formulating glyphosate did not reveal any adverse health effects. In operators applying glyphosate products, cases of eye, skin and/or respiratory irritation have been reported. Cases of acute intoxication have been observed after accidental or intentional ingestion of glyphosate formulations.

Summary

	Value	*Study*	*Safety factor*
ADI[a]	0–1.0 mg/kg bw	2-year study in rats (salivary gland effects)	100
ARfD	Unnecessary	—	—

[a] For the sum of glyphosate and AMPA

References

Acquavella, J.F., Alexander, B.H., Mandel, J.S., Gustin, C., Baker, B., Chapman, P. & Bleeke, M. (2004) Glyphosate biomonitoring for farmers and their families: results from the farm family exposure study. *Environ. Health Perspect.*, **112**, 321–326.

Acquavella, J.F., Weber, J.A., Cullen, M.R., Cruz, O.A., Martens, M.A., Holden, L.R., Riordan, S., Thompson, M. & Farmer, D.R. (1999) Human ocular effects from self-reported exposure to Roundup herbicides. *Hum. Exp. Toxcol.*, **18**, 479–486.

Allen, S.L. (1996) Glyphosate acid: Comparison of salivary gland effects in three strains of rat. Unpublished report No. CTL/P/5160, study No. PR 1029, dated 18 August 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Arbuckle, T.E., Burnett, R., Cole, D., Teschke, K., Dosemici, M., Bancej, C. & Zhang, J. (2002) Predictors of herbicide exposure in farm applicators. *Int. Arch. Occup. Environ. Health*, **75**, 406–414.

Atkinson, C., Perry, C.J., Hudson, P. & Snodgrass, E. (1989) Glyphosate: 4 week dietary toxicity study in rats. Unpublished report No. 5626, IRI project No. 437462, dated 18 January 1989, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Atkinson, C., Martin, T., Hudson, P. & Robb, D. (1993a) Glyphosate: 104 week dietary carcinogenicity study in mice. Unpublished report No. 7793, IRI project No. 438618, dated 12 April 1991, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Atkinson, C., Strutt, A.V., Henderson, W., Finch, J. & Hudson, P. (1993b) Glyphosate: 104 week combined chronic feeding/oncogenicity study in rats with 52 week interim kill (results after 104 weeks.). Unpublished report No. 7867, IRI project No. 438623, dated 7 April 1993, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Auletta, C.A. (1983) A dermal sensitization study in guinea pigs. Test material: glyphosate. Unpublished report, Bio/dynamics project No. 4235-82, Monsanto reference No. BD-83-008, dated 22 July 1983, from Bio/dynamics Inc., East Millstone, New Jersey, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Axelrad, J.C., Howard, C.V. & McLean, W.G. (2003) The effects of acute pesticide exposure on neuroblastoma cells chronically exposed to diazinon. *Toxicology*, **185**, 67–78.

Barbosa, E.R., Leiros de Costa, M.D., Bacheschi, L.A. & Scaff, M. (2001). Parkinsonism after glycine-derivate exposure. Mov. Disord., **16**, 565–568.

Blaszcak, D.L. (1988a) Acute oral toxicity study in rats. Test material: glyphosate wet cake. Unpublished report, Bio/dynamics project No. 4885-88, Monsanto reference No. BD-88-114, dated 20 September 1988, from Bio/dynamics Inc., East Millstone, New Jersey, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Blaszcak, D.L. (1988b) Acute dermal toxicity study in rabbits. Test material: glyphosate wet cake. Unpublished report, Bio/dynamics project No. 4886-88, Monsanto reference No. BD-88-114, dated 20 September 1988, from Bio/dynamics Inc., East Millstone, New Jersey, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Blaszcak, D.L. (1988c) Primary dermal irritation study in rabbits (4–hour exposure/semi-occlusive covering). Test material: glyphosate wet cake. Unpublished report, Bio/dynamics project No. 4887-88, Monsanto reference No. BD-88-114, dated 20 September 1988, from Bio/dynamics Inc., East Millstone, New Jersey, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Blaszcak, D.L. (1988d) Eye irritation study in rabbits. Test material: glyphosate wet cake. Unpublished report, Bio/dynamics project No. 4888-88, Monsanto reference No. BD-88-114, dated 20 September 1988, from Bio/dynamics Inc., East Millstone, New Jersey, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Blaszcak, D.L. (1998) MON 77945: primary eye irritation study in rabbits. Unpublished report, study No. 97-1728, Monsanto reference No. HU-97-242, dated 24 March 1998, from Huntingdon Life Sciences, East Millstone, New Jersey, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Bolognesi, C., Bonatti, S., Degan, P., Gallerani, E., Peluso, M., Rabboni, R., Roggieri, P. & Abbondandolo, A. (1997) Genotoxic activity of glyphosate and its technical formulation, Roundup. *J. Agric. Food Chem*., **45**, 1957–1962.

Bonnette, K.L. (2001) A primary eye irritation study in rabbits with MON 78623. Unpublished report, SLI study No. 3044.813, Monsanto study No. SB-2000-248, dated 6 August 1979, from Springborn Laboratories, Inc., Spencerville, Ohio, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Botham, P.A. (1996) First revision to glyphosate acid: 90 day feeding study in rats. Unpublished report No. CTL/P/1599, study No. PR 0663, dated 7 November 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Brammer, A. (1996) Glyphosate acid: 1 year dietary toxicity study in dogs. Unpublished report No. CTL/P/5079, study No. PD 1006, dated 24 September 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Brammer, A. (2001) Glyphosate acid: two year dietary toxicity and oncogenicity study in rats. Unpublished report No. CTL/PR1111, study No. PR1111, dated 15 March 2001, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Branch, D.K. (1981) Primary eye irritation of MON 0139 to rabbits. Unpublished report, study No. 800260, DMEH project No. ML-80-261, dated 17 March 1981, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Brooker, A.J., Brennan, C., John, D.M., Anderson, A. & Dawe, I.S. (1991a) The effect of glyphosate on pregnancy of the rabbit (incorporates preliminary investigations). Unpublished report No. CHV 45 & 39 & 40/901303, dated 14 October 1991, from Huntingdon Research Centre Ltd., Huntingdon, England. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Brooker, A.J., John, D.M., Anderson, A. & Dawe, I.S. (1991b) The effect of glyphosate on pregnancy of the rat (incorporates preliminary investigation). Unpublished report No. CHV 43 & 41/90716, dated 14 October 1991, from Huntingdon Research Centre Ltd., Huntingdon, England. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Brooker, A.J., Myers, D.P., Parker, C.A., Offer, J.M., Singh, H., Anderson, A. & Dawe, I.S. (1992) The effect of dietary administration of glyphosate on reproductive function of two generations in the rat. Unpublished report No. CHV 47/911129, dated 14 May 1992, from Huntingdon Research Centre Ltd., Huntingdon, England. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Busch, B. (1987a) Primary eye irritation study of MON-8722 in New Zealand white rabbits. Unpublished report, FDRL study No. 9307A, Monsanto study No. FD-86-430, dated 29 January 1987, from Food & Drug Research Laboratories, Waverly, New York, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Busch, B. (1987b) Primary eye irritation study of MON-8750 in New Zealand white rabbits. Unpublished report, FDRL study No. 9308A, Monsanto study No. FD-86-431, dated 30 January 1987, from Food & Drug Research Laboratories, Waverly, New York, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

California EPA (1996) California pesticide illness surveillance program report, 1994. Report HS-1733. Worker Health & Safety Branch, California Environmental Protection Agency, Department of Pesticide Regulation. Sacramento, California.

Callander, R.D. (1988) Aminomethyl phosphonic acid: An evaluation of the mutagenic potential using *S. typhimurium* and *E. coli.* Unpublished report No. CTL/P/2206, study No. YV2280 and YV2281, dated 21 September 1988, from ICI Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Callander, R.D. (1996) Glyphosate acid: An evaluation of mutagenic potential using *S. typhimurium* and *E. coli*. Unpublished report No. CTL/P/4874, study No. YV3611, dated 16 February 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Center de Toxicologie du Quebec (1988) Etude de l'exposition professionnelle des travailleurs forestiers exposés au glyphosate. Centre de Toxicologie du Quebec, August, 1988.

Chan, P.O. & Mahler, J.F. (1992) NTP technical report on toxicity studies of glyphosate (CAS No. 1071-83-6) administered in dosed feed to F344/N rats and B6C3F1 mice. National Toxicology Program, Research Triangle Park, NC, USA. NTP Toxicity Report Series No. 16, NIH Publication 92-3135, July 1992. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Chester, G. & Hart, T.B. (1986) Biological monitoring of a herbicide applied through backpack and vehicle sprayers. *Toxicol. Lett.*, **33**, 137–149.

Chruscielska, K., Graffstein, B., Szarapinska-Kwaszewska, J., Brzezinski, J. & Kalhorn, D. (2000) Glyphosate: evaluation of chronic activity and possible far-reaching effects. Part 2. Studies on mutagenic activity. *Pestycydy*, 3–4, 21–25.

Clay, P. (1996) Glyphosate acid: L5178Y TK$^{+/-}$ mouse lymphoma gene mutation assay. Unpublished report No. CTL/P/4991, study No. VV0123, dated 24 May 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Colvin, L.B. & Miller, J.A. (1973a) CP 67573 Residue and metabolism. Part 8: the gross metabolism of N-phosphonomethylglycine-14C (CP 67573-14C) in the laboratory rat following a single dose. Unpublished report No. 297, study No. 9-23-760.06-7863, dated 15 June 1973, from Monsanto Commercial Products Co., Agricultural Division, Research Department. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Colvin, L.B. & Miller, J.A. (1973b) CP 67573 Residue and metabolism. Part 9: the metabolism of *N*-phosphonmethylglycine-14C (CP 67573-14C) in the rabbit. Unpublished report No. 298, study No. 9-23-760.06-7863, dated 20 June 1973, from Monsanto Commercial Products Co., Agricultural Division, Research Department. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Cowell, J.E. & Steinmetz, J.R. (1990) Assessment of forestry nursery workers exposure to glyphosate during normal operations. Monsanto Report No. MSL-9655.

Curtis, K.M., Davitz, D.A., Weinberg, C.R. & Arbuckle, T.E. (1999) The effect of pesticide exposure on time to pregnancy. *Epidemiology*, **10**, 112–117.

Cuthbert, J.A. & Jackson, D. (1989a) Glyphosate technical: acute oral toxicity (limit) test in rats. Unpublished report No 5883, IRI project No. 243268, dated 22 June 1989, from Inveresk Research International, Musselburgh, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Cuthbert, J.A. & Jackson, D. (1989b) Glyphosate technical: primary skin irritation test in rabbits. Unpublished report No 5885, IRI project No. 243268, dated 18 June 1989, from Inveresk Research International, Musselburgh, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Cuthbert, J.A. & Jackson, D. (1989c) Glyphosate technical: primary eye irritation test in rabbits. Unpublished report No 5886, IRI project No. 243268, dated 1 July 1989, from Inveresk Research International, Musselburgh, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Cuthbert, J.A. & Jackson, D. (1989d) Glyphosate technical: Magnusson-Kligman maximisation test in guinea pigs. Unpublished report No 5887, IRI project No. 243268, dated 17 July 1989, from Inveresk Research International, Musselburgh, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Cuthbert, J.A. & Jackson, D. (1993a) AMPA: Acute oral toxicity (limit) test in rats. Unpublished report No 8763, IRI project No. 552409, dated 28 January 1993, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Cuthbert, J.A. & Jackson, D. (1993b) AMPA: acute dermal toxicity (limit) test in rats. Unpublished report No 8764, IRI project No. 552409, dated 28 January 1993, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Cuthbert, J.A. & Jackson, D. (1993c) AMPA: Magnusson-Kligman maximisation test in guinea pigs. Unpublished report No 8765, IRI project No. 552409, dated 28 January 1993, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Davies, D.J. (1996a) Glyphosate acid: excretion and tissue retention of a single oral dose (10 mg/kg) in the rat. Unpublished report No. CTL/P/4940, dated 26 April 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Davies, D.J. (1996b) Glyphosate acid: excretion and tissue retention of a single oral dose (1000 mg/kg) in the rat. Unpublished report No. CTL/P/4942, dated 19 June 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Davies, D.J. (1996c) Glyphosate acid: excretion and tissue retention of a single oral dose (10 mg/kg) in the rat following repeat dosing. Unpublished report No. CTL/P/4944, dated 22 May 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Davies, D.J. (1996d) Glyphosate acid: whole body autoradiography in the rat (10 mg/kg). Unpublished report No. CTL/P/4943, dated 10 June 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Dideriksen, L.H. (1991) Assessment of acute oral toxicity of "glyphosate technical" to mice. Unpublished report Lab. No. 12321, dated 7 January 1991, from Scantox A/S, Lille Skensved, Denmark. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Doyle, C.E. (1996a) Glyphosate acid: acute oral toxicity study in rats. Unpublished report No. CTL/P/4660, study No. AR5959, dated 23 August 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Doyle, C.E. (1996b) Glyphosate acid: acute dermal toxicity in the rat. Unpublished report No. CTL/P/4664, study No. CR3236, dated 23 August 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Doyle, C.E. (1996c) Glyphosate acid: skin irritation to the rabbit. Unpublished report No. CTL/P/4695, study No. EB4365, dated 23 August 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Doyle, C.E. (1996d) Glyphosate acid: skin sensitisation to the guinea pig. Unpublished report No. CTL/P/4699, study No. GG6427, dated 23 August 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Fox, V. (1998) Glyphosate acid: in vitro cytogenetic assay in human lymphocytes. Unpublished report No. CTL/P/6050, study No. SV0777, dated 29 October 1998, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Fox, V. & Mackay, J.M. (1996) Glyphosate acid: mouse bone marrow micronucleus test. Unpublished report No. CTL/P/4954, study No. SM0796, dated 21 March 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Franklin, C.A., Muir, N.I. & Moody, R.P. (1986) The use of biological monitoring in the estimation of exposure during the application of pesticides. *Toxicol. Lett*., **33**, 127–136.

Garry, V.F., Harkins, M.E., Erickson, L.L., Long-Simpson, L.K., Holland, S.E. & Burroughs, B.L. (2002) Birth defects, season of conception, and sex of children born to pesticide applicators living in the Red River Valley of Minnesota, USA. *Environ. Health Perspect*. **110** (suppl. 3), 441–449.

Goburdhun, R. (1991) Glyphosate: 52 week oral toxicity study in dogs. Unpublished report No 7502, IRI project No. 642675, dated 12 April 1991, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Goburdhun, R. & Oshodi, R.O. (1989) Glyphosate: oral maximum tolerated dose study in dogs. Unpublished report No 5660, IRI project No. 640683, dated 22 June 1989, from Inveresk Research International, Musselburgh, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Goldstein, D.A., Acquavella, J.F., Mannion, R.M. & Farmer, D.R. (2002) An analysis of glyphosate data from the California Environmental Protection Agency Pesticide Illness Surveillance Program. *J. Toxicol. Clin. Toxicol*., **40**, 885–892.

Hardell, L. & Erikson, M. (1999) A case-control study of non-Hodgkin lymphoma and exposure to pesticides. *Cancer*, **85**, 1353–1360.

Hazelden, K.P. (1992) AMPA: teratogenicity study in rats. Unpublished report No 7891, IRI project No. 490421, dated 29 December 1992, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Heath, J., Strutt, A. & Iswariah, V. (1993) AMPA: 4 week dose range finding study in rats with administration by gavage. Unpublished report No. 7803, IRI project No. 450860, dated 16 March 1993, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Heenehan, P.R. (1979a) Acute oral toxicity study in rats. Compound: glyphosate technical. Unpublished report No. BDN-77-428, Bio/dynamics project No. 4880-77, dated 6 August 1979, from Bio/dynamics Inc., USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Heenehan, P.R. (1979b) Acute dermal toxicity study [in rabbits]. Compound: glyphosate technical. Unpublished report No. BDN-77-428, Bio/dynamics project No. 4881-77, dated 6 August 1979, from Bio/dynamics Inc., USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Heenehan, P.R. (1979c) Primary dermal irritation study in rabbits. Compound: glyphosate technical. Unpublished report No. BDN-77-428, Bio/dynamics project No. 4883-77, dated 6 August 1979, from Bio/dynamics Inc., USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Heenehan, P.R. (1979d) Rabbit eye irritation study. Compound: glyphosate technical. Unpublished report No. BDN-77-428, Bio/dynamics project No. 4882-77, dated 6 August 1979, from Bio/dynamics Inc., USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Hodge, M.C.E. (1996) First revision to glyphosate acid: 90 day oral toxicity study in dogs. Unpublished report No. CTL/P/1802, study No. PD 0674, dated 14 November 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Horner, S.A. (1996a) Glyphosate acid: acute neurotoxicity study in rats. Unpublished report No. CTL/P/4866, study No. AR5968, dated 11 March 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Horner, S.A. (1996b) Glyphosate acid: subchronic neurotoxicity study in rats. Unpublished report No. CTL/P/4867, study No. PR1009, dated 11 March 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Howe, R.K., Chott, R.C. & McClanahan, R.H. (1988) The metabolism of glyphosate in Sprague-Dawley rats. Part II. Identification, characterisation and quantitation of glyphosate and its metabolites after intravenous and oral administration. Unpublished report No. MSL-7206, study No. 206300, dated February 1988, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, and Monsanto Life Sciences Research Center, Chesterfield, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Jauhiainen, A., Räsänen, K., Sarantila, R., Nuutinen, J. & Kangas, J. (1991) Occupational exposure of forest workers to glyphosate during brush saw spraying work. *Am. Ind. Hyg. Assoc. J.*, **52**, 61–64.

Jensen, J.C. (1991a) Mutagenicity test: Ames Salmonella assay with glyphosate, batch 206-JaK-25-1. Unpublished report Lab. No. 12323, dated 10 September 1991, from Scantox A/S, Lille Skensved, Denmark. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Jensen, J.C. (1991b) Mutagenicity test: in vitro mammalian cell gene mutation test with glyphosate, batch 206-JaK-25-1. Unpublished report Lab. No. 12325, dated 10 September 1991, from Scantox A/S, Lille Skensved, Denmark. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Jensen, J.C. (1991c) Mutagenicity test: micronucleus test with glyphosate, batch 206-JaK-25-1. Unpublished report Lab. No. 12324, dated 12 September 1991, from Scantox A/S, Lille Skensved, Denmark. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Jensen, J.C. (1993a) Mutagenicity test: Ames Salmonella test with AMPA, batch 286-JRJ-73-4. Unpublished report Lab. No. 13269, dated 18 February 1993, from Scantox A/S, Lille Skensved, Denmark. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Jensen, J.C. (1993b) Mutagenicity test: in vitro mammalian cell gene mutation test performed with mouse lymphoma cells (L5178Y). Test compound: AMPA, batch 286-JRJ-73-4. Unpublished report Lab. No. 13270, dated 18 February 1993, from Scantox A/S, Lille Skensved, Denmark. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Jensen, J.C. (1993c) Mutagenicity test: micronucleus test with AMPA, batch 286-JRJ-73-4. Unpublished report Lab. No. 13268, dated 18 February 1993, from Scantox A/S, Lille Skensved, Denmark. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Johnson, A.J. (1996) Glyphosate acid: acute delayed neurotoxicity study in the domestic hen. Unpublished report No. CTL/C/3122, project No. ISN 361/960244, dated 23 August 1996, from Huntingdon Life Sciences Ltd., Huntingdon, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Johnson, I.R. (1997) Glyphosate acid: eye irritation to the rabbit. Unpublished report No. CTL/P/5138, study No. FB5378, dated 18 March 1997, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Kaya, B., Creus, A., Yanikoglu, A., Cabré, O. & Marcos, R. (2000) Use of the *Drosophila* wing spot test in the genotoxicity testing of different herbicides. *Environ. Mol. Mutagen*., **36**, 40–46.

Kier, L.D. (1978) Final report on *Salmonella* mutagenicity assay of glyphosate. Unpublished report No. LF-78-161, dated 23 October 1978, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Kuhn, J.O. (1996) CHA 440: primary eye irritation study in rabbits. Unpublished report, study No 2981-96, dated 12 September 1996, from Stillmeadow Inc., Sugar Land, Texas, USA. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Lavy, T.L., Cowell, J.E., Steinmetz, J.R. & Massey, J.H. (1992) Conifer seedling nursery worker exposure to glyphosate. *Arch. Environ. Contam. Toxicol*., **22**, 6–13.

Leah, A.M. (1988) Aminomethyl phosphonic acid: acute oral toxicity to the rat. Unpublished report No. CTL/P/2266, study No. AR4690, dated 26 August 1988, from ICI Agrochemicals, Central Toxicology

Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Lee, H.I., Chen, K.W., Chi, C.H., Huang, J.J. & Tsai, L.M. (2000) Clinical presentations and prognostic factors of a glyphosate-surfactant herbicide intoxication: A review of 131 cases. *Acad. Emerg. Med*., **7**, 906–910.

Li, A.P. (1983a) CHO/HGPRT gene mutation assay with glyphosate. Unpublished report No. ML-83-155, study No. EHL 830079, dated 23 October 1978, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Li, A.P. (1983b) In vivo bone marrow cytogenetics study of glyphosate in Sprague-Dawley rats. Unpublished report No. ML-83-236, study No. EHL 830083, dated 20 October 1983, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Li, A.P. (1983c) Effects of glyphosate on rat bone marrow cells. Unpublished report No. ML-83-160, study No. EHL 830082, dated 21 October 1983, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Li, A.P. & Long, T.J. (1988) An evaluation of the genotoxic potential of glyphosate. *Fundam. Appl. Toxicol*., **10**, 537–546.

Lioi, M.B., Scarfi, M.R., Santoro, A., Barbieri, R., Zeni, O., Di Berardino, D. & Ursini, M.V. (1998a) Genotoxicity and oxidative stress induced by pesticide exposure in bovine lymphocyte cultures in vitro. *Mutat. Res*., **403**, 13–20.

Lioi, M.B., Scarfi, M.R., Santoro, A., Barbieri, R., Zeni, O., Salvemini, F., Di Berardino, D. & Ursini, M.V. (1998b) Cytogenetic damage and induction of pro-oxidant state in human lymphocytes exposed in vitro to gliphosate, vinclozolin, atrazine and DPX-E9636. *Environ. Mol. Mutagen*., **32**, 39–46.

Macpherson, D. (1996) Glyphosate acid: biotransformation in the rat. Unpublished report No. CTL/P/5058, dated 28 June 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Maibach, H.I. (1983) (a) Elimination of 14C-glyphosate in Rhesus monkeys following a single parenteral dose, (b) Percutaneous absorption of 14C-glyphosate in Roundup formulation in Rhesus monkeys following a single topical dose. Unpublished report No. MA-81-349, dated 1 April 1983, from University of California, School of Medicine; San Francisco, California, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

McDonald, P. & Anderson, B.T. (1989) Glyphosate technical: acute inhalation toxicity study in rats (limit test). Unpublished report No 5993, IRI project No. 642062, dated 25 August 1989, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

McDuffie, H.H., Pahwa, P., McLaughlin, J.R., Spinelli, J.J., Fincham, S., Dosman, J.A., Robson, D., Skinnider, L.F. & Choi, N.W. (2001) Non-Hodgkin's lymphoma and specific pesticide exposure in men: Cross-Canada study of pesticides and health. *Cancer Epidemiol. Biomark. Prev.*, **10**, 1155–1163.

Milburn, G.M. (1996) Glyphosate acid: one year dietary toxicity study in rats. Unpublished report No. CTL/P/5143, study No. PR 1012, dated 2 October 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Moxon, M.E. (1996a) Glyphosate acid: developmental toxicity study in the rat. Unpublished report No. CTL/P/4819, study No. RR0690, dated 27 March 1996, CTL/P/4819/amendment-001, dated 20 November 2002, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Moxon, M.E. (1996b) Glyphosate acid: developmental toxicity study in the rabbit. Unpublished report No. CTL/P/5009, study No. RB0709, dated 2 July 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Moxon, M.E. (2000) Glyphosate acid: multigeneration reproduction toxicity study in rats. Unpublished report No. CTL/P/6332, study No. RR0784, dated 16 June 2000, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Nordström, M., Hardell, L., Magnuson, A., Hagberg, H. & Rask-Andersen, A. (1998) Occupational exposures, animal exposure and smoking as risk factors for hairy cell leukaemia evaluated in a case-control study. *Br. J. Cancer*, **77**, 2048–2052.

Peluso, M., Munnia, A., Bolognesi, C. & Parodi, S. (1998) ^{32}P-postlabelling detection of DNA adducts in mice treated with the herbicide Roundup. *Environ. Mol. Mutagen*., **31**, 55–59.

Perry, C.J., Atkinson, C., Strutt, A., Henderson, W. & Hudson, P. (1991a) Glyphosate: 13 week dietary toxicity study in rats. Unpublished report No. 7136, IRI project No. 437876, dated 7 March 1991, from Inveresk Research International, Musselburgh, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Perry, C.J., Atkinson, C., Strutt, A., Hudson, P. & Jones, M. (1991b) Glyphosate: 13 week dietary toxicity study in mice. Unpublished report No 7024, IRI project No. 437918, dated 7 March 1991, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Petit, F., Le Goff, P., Cravedi, J.-P., Valotaire, Y. & Pakdel, F. (1997) Two complementary bioassays for screening the estrogenic potency of xenobiotics: recombinant yeast for trout estrogen receptor and trout hepatocyte cultures. *J. Molec. Endocrinol*., **19**, 321–335.

Powles, P. (1992a) 14C-glyphosate: absorption and distribution in the rat—preliminary study. Unpublished report No. 6365-676/1, dated 10 June 1992, from Hazleton UK, Harrogate, England. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Powles, P. (1992b) 14C-glyphosate: absorption, distribution, metabolism, and excretion in the rat. Unpublished report No. 7006-676/2, dated 30 June 1992, from Hazleton UK, Harrogate, England. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Powles, P. (1994a) 14C-glyphosate: absorption, distribution, metabolism and excretion following repeated oral administration to the dairy goat. Unpublished report No. 676/9-1011, dated 7 November 1994, from Hazleton Europe, Harrogate, England. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Powles, P. (1994b) 14C-glyphosate: distribution, metabolism and excretion following repeated oral administration to the laying hen. Unpublished report No. 676/8-1011, dated 7 November 1994, from Hazleton Europe, Harrogate, England. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Rattray, N.J. (1996) Glyphosate acid: 4-hour acute inhalation toxicity study in rats. Unpublished report No. CTL/P/4882, study No. HR2284, dated 29 April 1996, from Zeneca Agrochemicals, Central Toxicology Laboratory, Alderley Park, Macclesfield, Cheshire, England. Submitted to WHO by Syngenta Crop Protection AG, Basel, Switzerland.

Reagan, E.L. (1988a) Acute oral toxicity study of glyphosate batch/lot No. XLI-55 in Sprague-Dawley rats. Unpublished report, FDRL study No. 88.2053.007, Monsanto study No. FD-88-29, dated 8 June 1988, from Food & Drug Research Laboratories, Waverly, New York, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Reagan, E.L. (1988b) Acute dermal toxicity study of glyphosate batch/lot/nbr. No. XLI-55 in New Zealand White rabbits. Unpublished report, FDRL study No. 88.2053.008, Monsanto study No. FD-88-29, dated 8 June1988, from Food & Drug Research Laboratories, Waverly, New York, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Reagan, E.L. (1988c) Primary dermal irritation of glyphosate batch/lot/nbr No. XLI-55 in New Zealand White rabbits. Unpublished report, FDRL study No. 88.2053.010, Monsanto study No. FD-88-29, dated 8 June 1988, from Food & Drug Research Laboratories, Waverly, New York, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Reagan, E.L. (1988d) Primary eye irritation study of glyphosate batch/lot/nbr No. XLI-55 in New Zealand White rabbits. Unpublished report, FDRL study No. 88.2053.009, Monsanto study No. FD-88-29, dated 8 June1988, from Food & Drug Research Laboratories, Waverly, NY, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Reyna, M.S. & Thake, D.C. (1989) Range finding study of glyphosate administered in feed to Sprague-Dawley rats. Unpublished report No. ML-88-272, study No. 88181, dated April 1989, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Ridley, W.P. (1983) A study of the plasma and bone marrow levels of glyphosate following intraperitoneal administration in the rat. Unpublished report, study No. 830109, project No. ML-83-218, dated 24 October 1988, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Ridley, W.P. & Mirly, K. (1988) The metabolism of glyphosate in Sprague-Dawley rats. Part I. Excretion and tissue distribution of glyphosate and its metabolites following intravenous and oral administration. Unpublished report, study No. 86139, project No. ML-86-438, dated March 1988, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Rodwell, D.E. (1980) Dominant lethal study in mice. Unpublished report, study No. 401-064, IR-79-014, dated 16 April 1980, from International Research and Development Corporation, Mattawan, Michigan, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Rowe, L.D., Martin, B.W. & Peterson, H.D. (1987) The acute toxicity of glyphosate in female goats. Unpublished report, study No. 80006, Monsanto study No. VT-80-450, dated 23 March 1987, from U.S.D.A., Veterinary Toxicology and Entomology Research Laboratory, College Station, TX 77841, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Savitz, D.A., Arbuckle, T., Kaczor, D. & Curtis, K.M. (1997) Male pesticide exposure and pregnancy outcome. *Am. J. Epidemiol*., **146**, 1025–1036.

Sawada, Y., Nagai, Y., Ueyama, M. & Yamamoto, I. (1988) Probable toxicity of surface-active agent in commercial herbicide containing glyphosate. *Lancet*, **8580**, 299 (letter).

Shirasu, Y. & Takahashi, K. (1975) Acute toxicity of Roundup (correction: CP67573) in mice. Unpublished report No. ET-19-105, dated 5 March 1975, from Institute of Environmental Toxicology, Toxicology Division. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Shirasu, Y., Moriya, M. & Ohta, T. (1978) Microbial mutagenicity testing on CP67573 (glyphosate). Unpublished report No. ET-78-241, dated 20 July 1978, from Institute of Environmental Toxicology, Toxicology Division, Japan. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Stout, L.D. & Johnson, C.W. (1987) 90-day study of glyphosate administered in feed to Sprague/Dawley rats. Unpublished report No. ML-86-351, study No. EHL 86128, dated 30 November 1987, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Stout, L.D. & Ruecker, F.A. (1990) Chronic study of glyphosate administered in feed to albino rats. Unpublished report No. MSL-10495, job/project No. ML-87-148/EHL 87122, dated 22 October 1990, from Monsanto Environmental Health Laboratory, St. Louis, Missouri, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Strutt, A.V., Atkinson, C., Hudson, P. & Snodgrass, E. (1993) AMPA: 13 week toxicity study in rats with administration by gavage. Unpublished report No. 7866, IRI project No. 450876, dated 16 April 1993, from Inveresk Research International, Tranent, Scotland. Submitted to WHO by Cheminova A/S, Lemvig, Denmark.

Talbot, A.R., Shiaw, M.-H., Huang, J.-S., Yang, S.-F., Goo, T.-S., Wang, S.-H., Chen, C.-L. & Sanford, T.R. (1991) Acute poisoning with a glyphosate-surfactant herbicide ('Round-up'): A review of 93 cases. *Hum. Exp. Toxicol.*, **10**, 1–8.

Tominack, R.L., Conner, P. & Yamashita, M. (1989) Clinical management of Roundup herbicide exposure. *Jpn. J. Toxicol.*, **33**, 553.

Tominack, R.L., Yang, G.Y., Tsai, W.J., Chung, H.M. & Deng, J.F. (1991) Taiwan National Poison Center survey of glyphosate-surfactant herbicide ingestions. *Clin. Toxicol*., **29**, 91–109.

van de Waart, E.J. (1995) Evaluation of the ability of glyfosaat to induce chromosome aberrations in cultured peripheral human lymphocytes (with independent repeat). Unpublished report, Notox project No. 141918, dated 30 June 1995, from Notox B.V.'s-Hertogenbosch, Netherlands. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

Walsh, L.P., McCormick, C., Martin, C. & Stocco, D.M. (2000) Roundup inhibits steroidogenesis by disrupting steroidogenic acute regulatory (StAR) protein expression. *Environ. Health Perspect*., **108**, 769–776.

Williams, G.M., Kroes, R. & Munro, I.C. (2000) Safety evaluation and risk assessment of the herbicide Roundup and its active ingredient, glyphosate, for humans. *Regul. Toxicol. Pharmacol*., **31**, 117–165.

Williams, G.M. (1983) The hepatocyte primary culture/DNA repair assay on compound JJN-1020 (glyphosate) using rat hepatocytes in culture. Unpublished report No. AH-83-181, dated 21 October 1983, from Naylor Dana Institute for Disease Prevention, American Health Foundation, Valhalla, NY, USA. Submitted to WHO by Monsanto Int. Services SA, Brussels, Belgium.

PHORATE

First draft prepared by
P.K. Gupta
Toxicology Consulting Services Inc.,
Rajinder Nagar, Bareilly, Uta Pradesh, India

Explanation

Phorate is the International Organization for Standardization (ISO) approved name for phosphorothioic acid, *O*,-diethyl *S*-(ethyl thio)methyl ester, which is an organophosphate insecticide that inhibits acetylcholinesterase activity and is a systemic and contact insecticide and acaricide. Phorate was first evaluated by the Joint Meeting in 1977. In 1985, an acceptable daily intake (ADI) of 0–0.0002 mg/kg bw was established. Phorate was re-evaluated in 1994 when an ADI of 0–0.0005 mg/kg bw was established. In 1994, because it was reported in a limited study of metabolism in rats that <40% of the administered dose was excreted, the Meeting requested adequate studies on absorption, for review in 1996. Such studies were received and the ADI established previously was confirmed.

Since the 1994 JMPR, a study of acute neurotoxicity and a 13-week study of neurotoxicity in rats have been submitted. The present Meeting re-evaluated phorate within the periodic review programme of the Codex Committee on Pesticide Residues. The Meeting

considered new data that had not been reviewed previously and relevant data from previous evaluations.

Phorate is used against sucking and chewing insects, leafhoppers, leafminers, mites, some nematodes and rootworms, in order to protect a variety of crops, including corn, cotton, potatoes, tomatoes, sugar beets, edible beans, carrots, celery and peppers. Phorate is primarily formulated as granules to be applied at planting in a band or directly to the seed furrow. It is also used as a nematocide.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

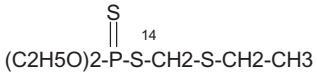
Rats

The 1994 review of studies of the metabolism of phorate indicated that male rats given ^{32}P-labelled phorate as a single oral dose at 2 mg/kg bw excreted 35% of the administered radiolabel in the urine and 3.5% in the faeces within 144 h. Male rats given six oral doses of phorate at 1 mg/kg bw per day excreted 12% of the administered radiolabel in the urine and 6% in the faeces within 7 days. Brain, liver, and kidney tissues from the latter animals contained unidentified, largely unextractable residues (Bowman & Casida, 1958).

Male rats were given a single dose of ^{14}C-labelled phorate (purity, >98%; specific activity, 40 µCi/mg; see Figure 1 for position of ^{14}C) at a dose of approximately 0.8 mg/kg bw by gavage in corn oil. Most of the administered dose was excreted in the urine (77%) and faeces (12%) within the first 24 h after dosing. Ninety-seven percent of the administered dose was recovered in the urine, faeces and cage rinses within 192 h (8 days). Peak tissue concentrations of total radioactive residue were found 6 h after treatment: blood, 0.37 ppm; kidney, 0.29 ppm; liver, 0.24 ppm in liver; skin, 0.20 ppm; muscle, 0.14 ppm; and fat, 0.08 ppm. The levels of residue declined throughout the course of the study, and by 48 h the concentrations of residue in muscle, fat, and skin were all <0.01 ppm. By 192 h, the residues in liver were only 0.02 ppm and those in kidney were <0.01 ppm. Approximately 80%, 79%, 84%, and 69% of the total residue present in urine, kidney, muscle and liver, respectively, was composed of a nonphosphorylated series of metabolites arising from the cleavage of the sulfur–phosphorus bond, methylation of the liberated thiol group and oxidation of the resulting sulfide to sulfoxide and sulfone. The remaining residue consisted of the phosphorylated metabolites. This study demonstrated that rats given phorate at a dose of 0.8 mg/kg bw quickly metabolize the parent compound and rapidly excrete the bulk of the biotransformed products, primarily in the urine (Hussain, 1987).

^{14}C-Labelled phorate (purity, >98% specific activity, 40 µCi/mg; see Figure 1 for position of radiolabel) was rapidly absorbed and excreted by female rats given a single oral dose at 0.44 mg/kg bw by gavage in corn oil. The urine was the primary route of elimination, and accounted for 78% of the administered dose within 24 h. Faecal elimination accounted for

Figure 1. Position of radiolabel in ^{14}C-labelled phorate



only 8% of the administered dose. Peak tissue levels of total radioactive residue were found after 6 h: blood, 0.168 ppm; kidney, 0.163 ppm; liver, 0.142 ppm; skin, 0.109 ppm; muscle, 0.100 ppm; and fat, 0.031 ppm. After 192 h (8 days), residues in the liver and kidney had depleted to only 0.008 and 0.010 ppm, respectively, while those in muscle, fat, skin, and blood were below the limit of detection of the assay by 48 h. After 192 h (8 days), 97% of the administered dose had been recovered in the urine, faeces and cage rinses. The results of this study indicate that phorate is rapidly absorbed and excreted by female rats. Greater than 94% of the administered dose was biotransformed to nonphosphorylated, non-toxicologically significant metabolites (Miller & Wu, 1990).

1.2 Biotransformation

Rats

The 1994 review of studies of the metabolism of phorate indicated that the urine of male rats given phorate at a daily dose of 1 mg/kg bw contained 17% diethyl phosphoric acid, 80% *O,O*-diethylphosphorothioic acid, and 3% *O,O*-diethyl phosphorodithioic acid. When ^{32}P-labelled phorate was incubated with prepared slices of rat liver, <1% of the radiolabelled compound was converted to hydrolysis products or unextractable residues. Phorate sulfoxide, phorate sulfone, phoratoxon sufoxide, and phoratoxon sulfone were formed (Bowman & Casida, 1958).

When a single dose of [^{14}C]phorate at 0.8 mg/kg bw was administered to male rats, the main urinary metabolites were the nonphosphorylated CL 180,298 (43%), CL 180,296 (28%), and CL 180,297 (9.6%) (see Figure 2 and Table 1 for identity of these metabolites). Phosphorylated metabolites (CL 18,061, CL 18,161, CL 18,162, CL 18,177, and CL 4,259) accounted for <15% of the recovered urinary metabolites, and the parent compound accounted for <1% of the administered dose. The main residues in liver, kidney, and muscle were also the nonphosphorylated metabolites, accounting for >68,79, and 83% of the tissue metabolites, respectively (Hussain, 1987).

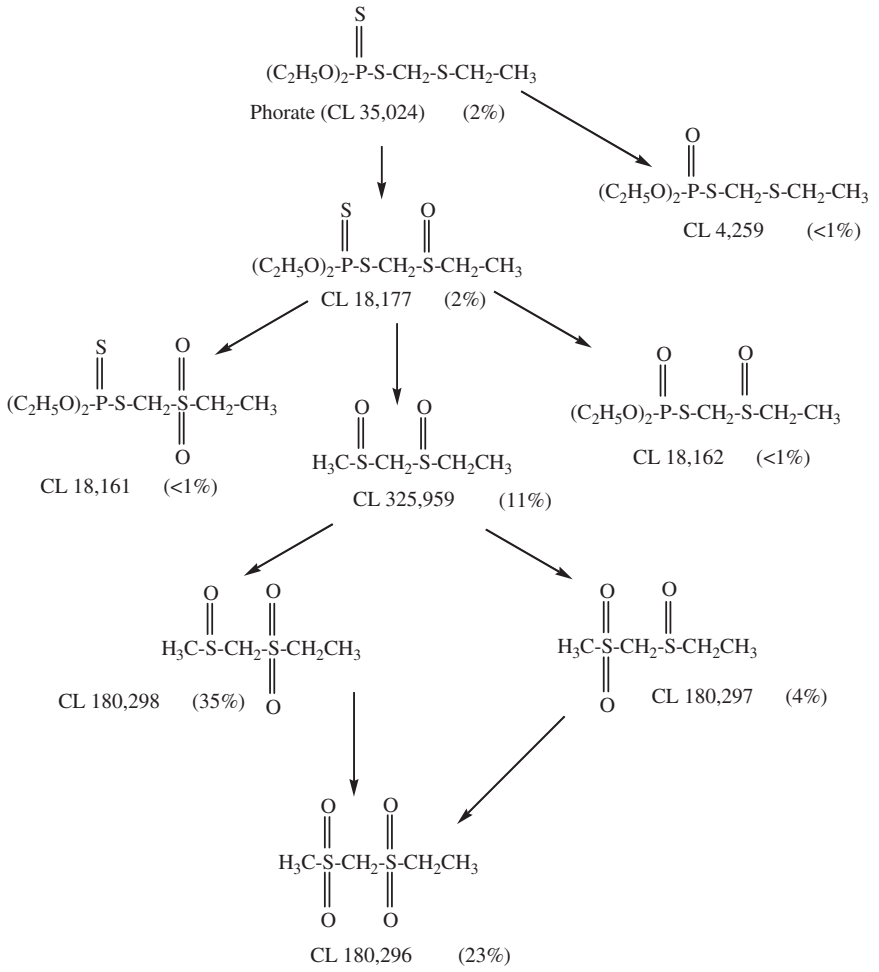
When female rats were given [^{14}C]phorate as a single dose at 0.44 mg/kg bw, the main urinary metabolites were the nonphosphorylated CL 180,298 (43%), CL 180,296 (24%), CL 325,959 (14%), and CL 180,297 (4.6%). No phosphorylated metabolites were identified in the tissues examined (liver, kidney and muscle). Two nonphosphorylated metabolites in the liver were identified as CL 180,286 (35%) and CL 180,298 <1%). Three metabolites in the muscle were identified as CL 180,296 (69.6%), CL 180,298 (7.0%) and CL 180,297

Table 1. Identification codes and chemical names of phorate and its metabolites

Identification code	Chemical name
Parent compound	
Phorate (CL 35,024)	Phosphorodithioic acid, *O*,*O*-diethyl *S*-(ethylthio)methylester
Metabolites	
CL 18,162	Phosphorothioic acid, *O*,*O*-diethyl *S*-(ethylsulfinyl)methylester
CL 18,177	Phosphorodithioic acid, *O*,*O*-diethyl *S*-(ethylsulfinyl)methylester "phorate sulfoxide"
CL 4,259	Phosphorothioic acid, *O*,*O*-diethyl *S*-(ethylthio)methylester
CL 18,161	Phosphorodithioic acid, *O*,*O*-diethyl *S*-(ethylsulfonyl)methylester "phorate sulfone"
CL 180,298	Sulfoxide, (ethylsulfonyl)methyl
CL 180,297	Sulfoxide, ethyl(methylsulfonyl)methyl
CL 180,296	Methane, (ethylsulfonyl)(methylsulfonyl)-
CL 352,959	Methane, (ethylsulfinyl)(methylsulfinyl)-

From Miller & Wu (1990)

Figure 2. Proposed metabolic pathway of phorate in rats



From Miller & Wu (1990)

(<1%). Two metabolites in the kidney were identified as CL 180,296 (22.4%) and CL 180,298 (1.7%). Similarly, no phosphorylated metabolites were identified in the urine. Faecal samples contained primarily the unchanged parent compound (33%) and the phosphorylated metabolites CL 18,177 (24%), CL 18,161 (8.8%), CL 18,162 (5.5%), and CL 4,259 (4.3%). The proposed metabolic pathway is presented in Figure 2, and the chemical names of the metabolites are given in Table 1 (Miller & Wu, 1990).

Phorate was rapidly absorbed and extensively detoxified by rats given a single oral dose by gavage. Urinary excretion was the principal route of elimination, accounting for approximately 80% of the administered dose within 24 h after dosing. Faecal excretion accounted for approximately 10% of the administered dose. After 192 h (8 days), essentially the entire administered dose had been eliminated by excretion.

The bulk of the administered dose (94%) was biotransformed to nonphosphorylated metabolites. The metabolic pathway for formation of these metabolites resulted from the cleavage of the phosphorus–sulfur bond, methylation of the liberated thiol group and oxidation of the resulting divalent sulfur moiety to the sulfoxide and sulfone. Thus, these studies demonstrated that phorate is rapidly absorbed and excreted and the accumulation of any toxic residue is not a concern.

1.3 Effects on enzymes and other biochemical parameters

Male Swiss albino mice were given phorate at a dose of 6728.5 mg/m^3 administered by inhalation using a whole-body inhalation chamber. Biochemical parameters were measured after 2, 4, 6, 8, 10, and 12 weeks of treatment. A dose-dependent significant decrease in pseudocholinesterase activity was observed throughout the experimental period. Concentration of haemoglobin, and erythrocyte count and erythrocyte volume fraction were significantly decreased from week 6 until the end of the experiment. During week 2, a significant increase in the total leukocyte count was observed, which was associated with an increase in monocyte and neutrophil counts. Subsequently, a significant decrease in counts was observed. The lungs of exposed animals showed a varying degree of bronchopneumonia and emphysema. After week 4, the animals slowly acclimatized and showed signs of recovery in haemoglobin and erythrocytes, while the leukocyte count decreased continuously. The sudy indicated that exposure to phorate leads to emphysematous changes and increased leukocyte count (Morowati, 1999).

The relative contribution of the cytochrome P450 (CYP)-dependent mono-oxygenase system and the flavo-containing monooxygenase in the microsomal oxidation of phorate were investigated in mice treated with phenobarbital, piperonyl butoxide, acetone, or hydrocortisone. Treatment with piperonyl butoxide produced a distinct biphasic effect (initial inhibition and subsequent induction) on the activities of several hepatic enzymes, including sulfoxidation of phorate. The relative contribution by CYP to phorate sulfoxidation decreased from 76% in the controls to 58% at 2 h after treatment and increased to 89% at 12 h. Treatment with hydrocortisone caused an increase in flavo-containing monooxygenase activity in the liver, but not in lung microsomes. Administration of acetone caused an increase in benzphetamine *N*-demethylation and *p*-nitrophenol hydroxylation in the liver, but no change in phorate sulfoxidase activity was observed, nor were the relative contributions attributable to CYP and flavo-containing monooxygenase altered. Treatment with phenobarbital produced a large increase in both benzphetamine *N*-dimethylase and phorate sulfoxidase activity in liver microsomes, with the percentage of the latter being increased due to CYP from 76% in the controls to 85% in the livers of treated rats (Kinsler, 1990).

2. Toxicological studies

2.1 Acute toxicity

(a) General toxicity

The results of studies of acute toxicity with technical-grade phorate are summarized in Table 2.

(b) Oral toxicity

The acute oral toxicity of technical-grade phorate was investigated in groups of male or female rats or mice (Table 2). The animals were observed for mortality and signs of toxicity for 14 days after administration. All the animals that received toxic or lethal doses showed typical clinical signs of cholinergic toxicity, such as salivation, lacrimation, exophthalmos, muscle fasciculation and excessive urination and defecation.

(c) Dermal toxicity

The acute dermal toxicity of technical-grade phorate was investigated in 10 Sprague-Dawley rats given single doses by dermal application (Newell & Dilley, 1978). Dilutions

Table 2. Results of studies of acute toxicity with technical-grade phorate

Species	Strain	Sex	Route and vehicle	LD_{50} (mg/kg bw)	LC_{50} (mg/l of air)	Reference
Mouse	NS	Male	Oral, in propylene glycol	2.25	—	Gaines (1969)
Mouse	NS	NS	Oral	11.00	—	Blinn (1982)
Mouse	NS	NS	Intraperitoneal	3.00	—	Blinn (1982)
Rat	Sherman	Male	Oral, in peanut oil	2.30	—	Gaines (1969)
Rat	Sherman	Female	Oral, in peanut oil	1.10	—	Gaines (1969)
Rat	Sprague-Dawley	Male	Oral, in propylene glycol	3.7	—	Newell & Dilley (1978)
Rat	Sprague-Dawley	Female	Oral, in propylene glycol	1.40	—	Newell & Dilley (1978)
Rat	Sprague-Dawley	Male	Oral	1.9–10	—	Blinn (1982)
Rat	NS	NS	Dermal	3.0	—	Blinn (1982)
Rat	Sprague-Dawley	Female	Dermal, in propylene glycol	3.90	—	Newell & Dilley (1978)
Rat	Sprague-Dawley	Male	Dermal, in propylene glycol	9.30	—	Newell & Dilley (1978)
Rat	Sherman	Male	Dermal, in xylene	6.20	—	Gaines (1969)
Rat	Sherman	Female	Dermal, in xylene	2.50	—	Gaines (1969)
Rat	Sprague-Dawley	Female	Intravenous	1.20	—	Newell & Dilley (1978)
Rat	Sprague-Dawley	Male	Intravenous	2.20	—	Newell & Dilley (1978)
Rat	Sprague-Dawley	Female	Inhalation	—	0.011 (1 h)	Newell & Dilley (1978)
Rat	Sprague-Dawley	Male	Inhalation	—	0.06 (1 h)	Newell & Dilley (1978)

NS, strains not specified

of the test substance were made in propylene glycol. The animals were observed for mortality and signs of toxicity for 14 days after administration. All the animals that received toxic or lethal doses showed typical clinical signs of cholinergic toxicity, such as salivation, lacrimation, exophthalmos, muscle fasciculation and excessive urination and defecation.

The dermal LD_{50} values for phorate were 9.3 (range, 7.9–11.0) mg/kg bw for males and 3.9 (range, 3.4–4.4) mg/kg bw for females.

(d) Exposure by inhalation

To determine the acute toxicity of technical-grade phorate administered by inhalation, groups of 10 male or female Sprague-Dawley rats were treated with an aerosol of phorate (technical grade, not diluted) by whole-body exposure (Newell & Dilley, 1978). The animals were observed for mortality and signs of toxicity during exposure and for 14 days after administration. All the animals that received toxic or lethal doses showed typical clinical signs of cholinergic toxicity, such as salivation, lacrimation, exophthalmos, muscle fasciculation and excessive urination and defecation.

The median lethal concentration (LC_{50}) values for an exposure of 1 h were calculated to be 0.06 (range, 0.052–0.069) mg/l of air for males and 0.011 (range, 0.007–0.015) mg/l of air for females.

(e) Dermal and ocular irritation and dermal sensitization

Since technical-grade phorate is highly toxic by dermal contact, dermal irritation potential could not be determined.

Since technical-grade phorate is highly toxic by dermal contact, ocular irritation potential could not be determined.

Because of the marked acute toxicity of technical-grade phorate by dermal contact, a study of dermal sensitization was not performed.

2.2 Short-term studies of toxicity

Mice

In a study that complied with the principles of good laboratory practice (GLP) and was certified for quality assurance (QA), groups of 20 male and 20 female Crl:CD1®(ICR)BR (outbred Swiss albino mice) were given access ad libitum to diets containing technical-grade phorate (purity, 92.1%) at a concentration of 0, 1, 3, or 6 ppm for 13 weeks. Terminal sacrifice was performed during week 14. Survival, clinical signs, body weights, food consumption, findings after necropsy for an unscheduled death, and terminal studies of clinical pathology (plasma, erythrocyte, and brain cholinesterase activities) were evaluated. Histopathological examinations were not performed.

The mean daily intake of phorate is summarized in Table 3.

There were no toxic effects evident in any of the treated groups of mice in terms of survival rates (survival/adjusted survival was 100%), body weight and food consumption measurements, or clinical and necropsy observations. At 6 ppm, one male convulsed for about 20 s when placed in the weighing pan at week 13. Similar findings occasionally occur in untreated mice in this laboratory and are thought to represent spontaneous epileptic seizures. The relationship, if any, of this isolated finding to treatment in this study is not known.

Terminal studies of clinical pathology showed inhibition of cholinesterase activities in females at 1 ppm and in males and females at 3 and 6 ppm. Apparent effects were greater on the plasma than on erythrocytes or the brain. Plasma cholinesterase activities were below those of controls for females at 1 ppm (15%), males (47%) and females (61%) at 3 ppm and males (82%) and females (88%) at 6 ppm. Erythrocyte cholinesterase activities were decreased by 17% for females at 3 ppm and by 50% and 61% for males and females at 6 ppm. These blood values were significantly decreased (except for the erythrocyte cholinesterase activity of females at 3 ppm) as were brain cholinesterase activities at the two highest dietary concentrations—in males and females at 3 ppm (left half of the brain showed a 12% decrease for males; right and left brain halves showed decreases of 9% and 13% for females) and in males and females at 6 ppm (decreases of 49% and 50% in males, and 53% and 54% in females, for left and right brain halves, respectively).

In conclusion, under the conditions of this study, administration of diets containing phorate at a concentration of 1, 3, or 6 ppm to Swiss albino mice for 13 weeks produced dose-related inhibition of cholinesterase activity, but no other apparent signs of toxicity. Effects on plasma cholinesterase activity exceeded those observed for erythrocyte or brain cholinesterase activity.

Table 3. Mean daily substance intake in mice fed diets containing phorate for 13 weeks

Dietary concentration (ppm)	Mean daily intake (mg/kg bw)	
	Males	Females
1	0.18	0.23
3	0.55	0.67
6	1.10	1.38

From Trutter (1990)

On the basis of inhibition of erythrocyte and brain cholinesterase, the lowest-observed-adverse-effect level (LOAEL) was 6 ppm (equal to an intake of 1.10 mg/kg bw per day), supporting a no-observed-adverse-effect level (NOAEL) of 3 ppm (equal to 0.55 mg/kg bw per day). The 9% and 17% decreases in erythrocyte and brain cholinesterase activities, respectively, at 3 ppm were not considered to be toxicologically significant (Trutter, 1990).

Rats

In a 13-week study, groups of 50 male and 50 female (control and groups B, C, D and E) or 25 male and 25 female (groups F and G) albino Carworth Farms rats were given access ad libitum to diets containing technical-grade phorate (purity, 92%) at a concentration of 0, 0.22, 0.66, 2.0, 6.0, 12.0 or 18.0 ppm (equivalent to 0, 0.011, 0.033, 0.1, 0.3, 0.6 or 0.9 mg/kg bw per day respectively) for 13 weeks.

Males and females at 12 and 18 ppm showed severe excitability, intermittent tremors and ataxia, which resulted in the death of 50% of the animals at 12 ppm and no survivors at 18 ppm. Occasional episodes of excitability, intermittent tremors were noted in females at 6 ppm. In all groups receiving phorate at ≤6 ppm, survival, body-weight gain, food consumption and liver and kidney weights were within normal limits.

Erythrocyte cholinesterase activity was inhibited (by approximately 30%) in females at 2 ppm, while at 6 ppm, plasma, erythrocyte and brain cholinesterase activities were inhibited in both sexes (brain cholinesterase by approximately 85% in females).

No adverse effects were observed at gross necropsy or histopathological examination.

The NOAEL was 2 ppm (equal to 0.1 mg/kg bw per day) on the basis of inhibition of brain acetylcholinesterase activity at 6 ppm (equal to 0.3 mg/kg bw per day) (Tusing et al., 1956b).

Dogs

Groups of two male and one female mongrel dogs received capsules containing technical-grade phorate (purity, 92%; in corn oil) at a dose of 0, 0.01, 0.05, 0.25 or 1.25 mg/kg bw per day, 6 days per week for 15 weeks. Two males received a single dose of 2.5 mg/kg bw per day. Plasma and erythrocyte cholinesterase activities were measured weekly; brain acetylcholinesterase activity was not measured. At 0.05 mg/kg bw per day, plasma cholinesterase activity was significantly depressed. Erythrocyte cholinesterase activity at 0.05 mg/kg bw per day was not affected during the first 12 weeks of the study, but was depressed slightly, not significantly, during the last 3 weeks of the study. Significant decreases in plasma and erythrocyte acetylcholinesterase activity were observed at 0.25 mg/kg bw per day; total inhibition of plasma cholinesterase activity and a significant reduction in erythrocyte cholinesterase activity were noted at 1.25 mg/kg bw per day. All the dogs receiving phorate as a single dose at 2.5 mg/kg bw per day died within 3–4 h; cholinesterase activity was not determined. No signs of systemic toxicity and no adverse effects on haematological, clinical chemistry or urine analysis parameters were observed in dogs at 0.01 and 0.05 mg/kg bw per day. Histopathological examination revealed no consistent treatment-related findings.

The NOAEL was 0.05 mg/kg bw per day on the basis of inhibition of erythrocyte cholinesterase activity at higher doses (Tusing et al., 1956a).

Groups of three male and three female beagle dogs were fed diets containing technical-grade phorate at a concentration of 0, 0.5 or 1.0 ppm, equivalent to 0.012 or 0.025 mg/kg bw per day, for 6 weeks. Cholinesterase activity in plasma and erythrocytes was determined before study initiation and every 2 weeks during the study. No significant differences in plasma and erythrocyte cholinesterase activities were noted between the treated and control animals (Kay & Calandra, 1961).

In a 14-day study designed to evaluate the toxicity of technical-grade phorate administered orally, five groups (groups 2 to 6) of two male and two female beagle dogs received capsules containing phorate (purity, 92.1%) at a dose of 0.01, 0.05, 0.1, 0.25 or 0.5 mg/kg bw per day for 14 consecutive days. A control group (group l, comprising three males and three females) was given capsules containing corn oil. Groups 4 to 6 began the study 1 week after groups 1 to 3. The dogs were observed for mortality and signs of pharmacotoxicity four times daily. Food consumption was measured and recorded daily. Body weights were recorded on days 1 and 8, and at terminal sacrifice. Blood biochemical and haematological parameters were determined for each dog during the pre-test period and before study termination. Special assays for erythrocyte and plasma cholinesterase activities were performed twice for each dog during the pre-test period and after 3, 7, 10 and 14 doses of the test substance. Brain acetylcholinesterase activity was determined in the cerebellum and cerebrum of each dog after termination of the study. All surviving animals were necropsied at termination.

Excessive salivation and tremors seen in animals at the highest dose were probably related to administration of the test substance, while salivation seen at 0.05 mg/kg bw per day was probably a random occurrence. A slight decrease in body-weight gain in animals at the highest dose was probably related to administration of phorate. No definitive treatment-related effects were observed on food consumption, haematology, or organ weights. Necropsy did not reveal any gross lesions in any of the treated dogs. A depression in serum protein values was observed at 0.5 mg/kg bw per day in both males and females. Plasma cholinesterase activity was inhibited at doses of ≥0.05 mg/kg bw per day in males and at ≥0.1 mg/kg bw per day in females. Cholinesterase activity in the brain (cerebellum) was inhibited (by 31–69%) in males and females at doses of ≥0.1 mg/kg bw per day. Erythrocyte cholinesterase activity was slightly inhibited (by approximately 25%) in males and females at the highest dose tested, 0.5 mg/kg bw per day. These findings were considered to be treatment-related.

The NOAEL was 0.05 mg/kg bw per day on the basis of inhibition of brain acetylcholinesterase activity (Piccirillo et al., 1987).

Dogs

In a 1-year study of oral toxicity, groups of purebred beagle dogs (aged 6 months) were given capsules containing technical-grade phorate (purity, 92.1%) at a dose of 0, 0.005, 0.01, 0.05 or 0.25 mg/kg bw per day. The treated groups comprised six animals of each sex per group while the control group comprised eight animals of each sex. All animals were observed twice daily for overt signs of toxicity; detailed examinations were conducted weekly. Body weights were recorded before testing, at the start of the dosing period, weekly thereafter throughout the study, and at terminal sacrifice. Food consumption was determined daily throughout the study. Blood biochemical, haematological and urine analysis parameters and plasma and erythrocyte cholinesterase activities were determined before testing, at

6 weeks, 3, 6 months and at study termination. Acetylcholinesterase activities in the cerebrum and cerebellum were determined after termination of the study.

Clinical signs indicative of cholinergic toxicity (mild tremors) were seen occasionally in males and females at the highest dose (0.25 mg/kg bw per day). One male at 0.01 mg/kg bw per day was sacrificed in a moribund condition during week 24, but this was considered to be incidental to treatment. All other animals survived to the terminal necropsy with no consistent clinical signs that could be attributed to the administration of phorate. Mean body weights of males at the highest dose were consistently lower than those of the controls, but the differences were not statistically significant; mean body weights of females at all doses were similar to those of the controls throughout the study. Food consumption of treated males and females at all doses were similar to those of the controls throughout the study. Ophthalmological examinations revealed no treatment-related effects. Examinations of haematology, clinical chemistry parameters and urine analysis revealed no biologically significant changes.

At 0.01 mg/kg bw per day, there were marginal effects on plasma cholinesterase activity in males and females. However, statistically significant inhibition of plasma cholinesterase activity (25–80%) was observed in males and females at doses of 0.05 and 0.25 mg/kg bw per day. The highest dose, 0.25 mg/kg bw per day, resulted in reduced erythrocyte (20% inhibition) and brain cholinesterase (43–54% inhibition) activities in males and females.

The overall NOAEL was 0.05 mg/kg bw per day on the basis of decreased body weight, significant inhibition of erythrocyte and brain acetylcholinesterase activity and clinical signs consistent with cholinergic toxicity at the highest dose of 0.25 mg/kg bw per day (Shellenberger & Tegeris, 1987).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

The long-term toxicity and oncogenic potential of technical-grade phorate was evaluated in a 18-month study in mice. Groups of 50 male and 50 female CD1 outbred Swiss albino mice were given diets containing phorate (purity, 91.7%) at a concentration of 0, 1, 3 or 6 ppm (equal to 0, 0.15, 0.45 and 0.9 mg/kg bw per day). The animals were observed before the start of the study and at least daily thereafter for general physical appearance, mortality and signs of toxicity. Each animal was removed from the cage and examined weekly in detail for clinical signs, including palpation for masses. Body weight was measured before the start of the study; body weights and food consumption were subsequently obtained at weekly intervals through week 13, biweekly for weeks 15–25 and monthly until termination. Animals found dead or moribund and that were sacrificed during the study were necropsied and a spectrum of the tissues were preserved in 10% formalin for future evaluation. At 18 months, all remaining animals were killed and a gross necropsy was performed on each animal and tissues were preserved in 10% buffered formalin. Tissues saved from all animals were examined histopathologically.

During the course of the study, 91 mice died or were killed in a moribund condition, as summarized in Table 4.

Table 4. Mortality to study termination in mice fed diets containing phorate for 18 months

Dietary concentration (ppm)	Cumulative mortality (%)	
	Males	Females
0	5/50 (10%)	13/50 (26%)
1	7/50 (14%)	13/50 (26%)
3	11/50 (22%)	16/50 (49)[a] (32% or 32.7%)
6	9/50 (18%)	17/50 (49)[a] (34% or 34.7)

From Manus et al. (1981a)
[a] The totals shown above reflect situations in which mice were missing

None of the deaths were attributed to the effects of treatment as mortality was similarly distributed throughout control and treated groups.

Some clinical signs, such as tremors, hyperactivity and excessive salivation, occurred at a higher incidence and more frequently in animals fed with diets containing phorate at 6 ppm than in animals in the control groups.

The only treatment-related effect was a reduction in the mean body weight of females at 6 ppm throughout the study, with the differences (compared with controls) at 13 separate time-points being statistically significant. While there was a suggestion of a similar trend in males at 6 ppm, only at three early time-points were the differences statistically significant. Otherwise, the body weights of the treated animals were comparable to those of animals in the control group. Differences in mean body weight did not seem to be caused by reduced consumption of food. All treated animals appeared to eat less during the first 3 weeks and occasionally thereafter, but no consistent dose–response relationship was seen.

Gross pathological examination showed no changes that were significantly different from those in animals in the control groups, and histopathological examination revealed no alterations that were related to treatment. There was no significant dose-related increase in the incidence of any particular type of tumour, of animals with tumours, of animals with malignant tumours or of animals with multiple primary tumours. The NOAEL was 3 ppm (equal to 0.45 mg/kg bw per day) on the basis of decreased body weight and clinical signs of toxicity at 6 ppm (equal to 0.90 mg/kg bw per day) (Manus et al., 1981a).

Rats

In a long-term study of oral toxicity and potential carcinogenicity, groups of 50 male and 50 female Crl:COBS CD(SD)BR rats were given diets containing technical-grade phorate (purity, 91.7%) at a concentration of 0, 1, 3 or 6 ppm (equal to 0.05, 0.16 or 0.32 mg/kg bw per day in males and 0.07, 0.19 or 0.43 mg/kg bw per day in females) for 24 months. The parameters evaluated in this study were clinical signs, body weight, food consumption, haematology, clinical chemistry, urine analysis, and plasma, erythrocyte and brain cholinesterase activity; gross necropsy and histopathological evaluations were carried out at study termination.

The numbers of rats in each group that survived to the end of the study are summarized in Table 5.

Table 5. Survival to study termination in rats fed diets containing phorate for 24 months

Dietary concentration (ppm)	No. of rats that survived to study termination (%)	
	Males	Females
0	29/51 (57%)	28/50 (56%)
1	27/50 (54%)	24/50 (48%)
3	27/50 (54%)	34/50 (68%)
6	24/50 (48%)	18/50 (36%)

From Manus et al. (1981b)

The only clinical sign related to treatment was tremors induced by over-dosing (327% of all the intended doses) during week 9. Growth was depressed in females at 6 ppm during the first 26 weeks and again between weeks 74 and 102. Food consumption showed no consistent dose–response pattern. On haematological examination, clinical chemistry and urine analysis performed at 6, 12 and 24 months, the only notable findings were decreased values for erythrocyte counts, haemoglobin and erythrocyte volume fraction in females at the highest dose after 12 months.

Dose-related inhibition (>20%) of plasma cholinesterase activity was noted in males at 6 ppm at 12 months, in all treated males at 24 months and in females at both 3 and 6 ppm at all time-points (3, 6, 12 and 24 months). Erythrocyte acetylcholinesterase was not significantly depressed (<20%) at any time. The activity of brain acetylcholinesterase was reduced (>20%) in males at 6 ppm and in females at 3 and 6 ppm.

At sacrifice, females at 6 ppm had increased organ : body weight ratios with respect to the adrenals, brain, heart, liver and spleen. On gross pathological and histopathological examination, the only finding that could possibly be attributed to test substance administration was the slight increase in the incident of inflammation and epithelial hyperplasia of the forestomach in both males and females, but particularly in males at 6 ppm. Similar lesions were seen in animals in the control group, and have been reported to be a relatively common incidental finding in laboratory rats. There was no obvious trend in incidence of the lesions in the groups receiving the intermediate dose. For these reasons, the increased incidence of forestomach lesions in treated rats was considered to be attributable to random variation, or, at most, irritation of forestomach. There were no significant differences between control and treated groups with regard to incidence, type or time of appearance of tumours.

The NOAEL was 1 ppm (equal 0.07 mg/kg bw per day) on the basis of inhibition of brain acetylcholinesterase activity in females at 3 ppm (equal to 0.19 mg/kg bw per day) (Manus et al., 1981b).

2.4 Genotoxicity

The results of studies of genotoxicity with phorate are summarized in Table 6. Phorate was not found to be genotoxic in vitro or in vivo.

Table 6. Results of studies of genotoxicity with phorate

End-point	Test object	Concentration or dose	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA100, TA1535, TA1537, TA1538; *E. coli* WP2	≤1000 mg/plate	Technical grade	Negative[a]	Allen (1978)
Reverse mutation	*E. coli* p3478, W3110; *B. subtilis*	1 mg (on filter disc) per plate	85	Negative[b]	Simmon et al. (1977)
Reverse mutation	Chinese hamster ovary cells, *Hprt* locus	30, 40, 50, 80, or 100 nl/ml	92.1	Negative[b]	Thilagar & Kumarop (1985)
		5, 10, 12, 14, 16, or 18 nl/ml		Negative[c]	
Mitotic recombination	*S. cerevisiae* D3	5% w/v for 4 h incubation before plating	85	Negative[a]	Simmon et al. (1977)
Unscheduled DNA synthesis	Human fibroblasts WI-38	$\leq 1 \times 10^{-3}$	85	Negative[a]	Simmon et al. (1977)
In vivo					
Chromosomal aberration	Male and female Sprague-Dawley rats killed after 6, 18, or 30 h	Males: 0 (corn oil), 0.25, 1.25, or 2.5 mg/kg bw per day; Females: 0, 0.13, 0.63, or 1.25 mg/kg bw per day	92.1	Negative	Ivett & Myhr (1986)
Dominant lethal mutation	Male mice	0, 5, 10, or 20 mg/kg bw per day in diet for 7 weeks, weekly matings for 8 weeks	85	Negative	Simmon et al. (1977)

[a] In the presence and absence of metabolic activation
[b] In the absence of metabolic activation
[c] In the presence of metabolic activation

Table 7. Mean daily substance intake in mice fed diets containing phorate for 3 weeks

Dietary concentration (ppm)	Mean daily intake (mg/kg bw)	
	Males	Females
0.6	0.12	0.13
1.5	0.30	0.33
3.0	0.54	0.64

From Morici et al. (1965)

2.5 Reproductive toxicity

(a) Multigeneration study

Mice

Groups of eight male and 16 female CF1 mice were fed diets containing technical-grade phorate (purity, 98.4%) at a concentration of 0, 0.6, 1.5 or 3 ppm for three generations, with two litters per generation. Food intake was measured only for the F_0 generation before the initial mating. Since animals were housed in pairs, no measurement of individual consumption was obtained. The mean intakes of phorate for the 3-week period are summarized in Table 7.

There were no dose-related effects on indices of fertility, gestation, viability or lactation during the study, but the lactation index was lowered in four of the six litters in the group of animals receiving phorate at 3 ppm, to below the value for control animals in the first mating of the F_0 generation, in both matings of the F_1 generation and in the second mating of the F_2 generations. Gross and microscopic examination of tissues revealed no treatment-related effects.

The NOAEL was 1.5 ppm (equal to 0.30 mg/kg bw per day for males and 0.33 mg/kg bw per day for females) on the basis of decreased lactation indices at 3 ppm (Morici et al., 1965).

Rats

In a study that complied with the principles of GLP and that was certified for QA, reproductive performance and fertility were observed through two generations of rats that were given diets containing phorate (purity, 92.1%) at a concentration of 1, 2, 4 or 6 ppm. Each parent generation (P_1 and F_1), consisted of groups of 25 male and 25 female COBS CD®(SD) rats, except for the F_1 generation at 6 ppm that consisted of 30 males and 30 females at study initiation. Also included was a diet control group of 25 males and 25 females. Animals of the P_1 generation received at least 60 days of treatment before initiation of mating to produce the first litters, while animals of the F_1 generation received at least 100 days treatment before initiation of mating. During these pre-mating periods of treatment, body weights and food consumption were recorded weekly. Animals of the parental generation received a detailed physical examination weekly throughout the study. Body weights of mated females were recorded during gestation and lactation, and food consumption was recorded during gestation.

Each parental generation was mated to produce two litters. Randomly selected offspring from the second litter (F_{1b}) of the P_1 generation were chosen to become the parents of the ensuing generation. Offspring not included in the selection procedure and offspring from the first litter of each generation (F_1, F_{2a}) and the F_{2b} offspring were given a gross external examination and discarded. Additionally, randomly selected offspring (one of each sex per litter) from the second litters of the P_1 and F_1 generations (F_{1b} and F_{2b} litters, respectively) were sacrificed and given a gross postmortem examination (abnormal tissues were saved in 10% formalin). Animals of the P_1 and F_1 adult generations were sacrificed, given a gross postmortem evaluation, and pituitary glands and reproductive tissues/gross lesions were taken and preserved in 10% neutral buffered formalin. The eyes from all P_1 parental females, and the eyes plus the intra- and extra-ocular muscles and optic nerve from all the F_1 parental animals were preserved in 10% formalin. Reproductive tissues and pituitary glands were processed for histopathological evaluations for the P_1 and F_1 animals in the control group and the group receiving phorate at a dietary concentration of 6 ppm, and ocular tissues, including the intra- and extra-ocular muscles, retina and optic nerve, were evaluated for all animals in the control group and treated F_1 parental animals. Additionally, in the F_1 parental animals, plasma, erythrocyte and brain cholinesterase activities were determined for 10 randomly selected animals of each sex per group at terminal sacrifice. Ophthalmoscopic evaluations were conducted for females of the P_1 parental generation before scheduled sacrifice and for all animals of the F_1 parental generation at initiation of the pre-mating period of treatment and again several weeks before scheduled sacrifice.

Mean daily intakes of the test substance for the treated groups, calculated from data on mean weekly food consumption during the pre-mating periods, are summarized in Table 8.

Treatment with phorate at a dietary concentration of 1 or 2 ppm produced no adverse effects in animals of the parental generation (P_1, F_1) with regard to the following parameters: growth during the pre-mating period of treatment; food consumption; physical observations; maternal-weight gain during gestation/lactation; maternal food consumption during

Table 8. Mean daily substance intake in a study of reproductive toxicity in rats fed diets containing phorate

Dietary concentration (ppm)	Mean daily intake (mg/kg bw)			
	P_1		F_1	
	Males	Females	Males	Females
1	0.086	0.101	0.088	0.105
2	0.171	0.197	0.181	0.205
4	0.347	0.401	0.370	0.439
6	0.523	0.622	0.683	0.831

From Schroeder (1991)

Table 9. Differences in mean cholinesterase activity in F_1 parental rats fed diets containing phorate compared with controls

Dietary concentration (ppm)	Difference (%) in mean cholinesterase activity					
	Males			Females		
	Plasma	Erythrocyte	Brain	Plasma	Erythrocyte	Brain
1	+20.8	+1.3	+3.6	+3.3	−2.7	−1.2
2	−2.2	0	+2.4	−19.2	−1.4	−17.4
4	−25.2	−6.3	−14.5	−74.4**	−4.1	−59.3**
6	−40.3**	−10.1*	−39.8**	−95.8**	−11.0	−82.6**

From Schroeder (1991)
*$p > 0.05$; **$p > 0.01$; statistically significant differences from data for controls

gestation; reproductive performance/fertility indices; duration of gestation; and parturition. For each litter interval, pup weights, pup sex distribution indices, pup survival indices during lactation and pup external examinations indicated no adverse effects of treatment at dietary concentrations of ≤2 ppm.

Ophthalmoscopic evaluations of the P_1 females and F_1 males and females and gross postmortem evaluations of animals of the parental generation at these same dietary concentrations (1 and 2 ppm) also demonstrated no adverse effects of treatment. Cholinesterase activities (plasma, erythrocyte and brain) were unaffected by treatment at 1 ppm. Although slight reductions in plasma and brain cholinesterase activities (19.2% and 17.4%, respectively) were seen in F_1 females at 2 ppm, these changes were not statistically significant from values for the controls and were not considered to be physiologically significant. Cholinesterase activities for males at 2 ppm were comparable to those of the controls (Table 9).

At 4 ppm, the following treatment-related responses were seen: tremors in several females from each parental generation; body-weight loss for days 0–21 of lactation (consistent for both litter intervals of each parental generation); reduction of plasma (74% inhibition) and brain (59% inhibition) cholinesterase activities for F_1 females; and reduced pup weights on days 14 and 21 of lactation (both litter intervals of the P_1 generation and the F_{2a} litters). Pup survival indices for days 0–4 and 4–21 of lactation were lower than those for controls at 4 ppm only for the F_{2a} litters. No adverse effects on body weight (other than weight loss during lactation), food consumption or mating/pregnancy/fertility indices and parturition were seen at 4 ppm. Also, ophthalmoscopic evaluations of the F_1 parental animals at 4 ppm revealed no treatment effects.

In the group receiving phorate at a dietary concentration of 6 ppm, the following treatment-related effects were seen: increased mortality in animals of the F_1 parental generation, particularly early in the pre-mating interval; tremors in the P_1 females and F_1 males and females; reduced body weights during the pre-mating periods for the P_1 females and F_1 males and females; reduced body-weight gain over the entire pre-mating interval for the P_1 females; reduced gestation/lactation weights (both litters of each generation); reduction in plasma (40–96% inhibition), erythrocyte (10% inhibition) and brain (40–83% inhibition) cholinesterase activities for the F_1 parental animals; reduced litter size at birth for the F_{2a} and F_{2b} litters of the F_1 generation; reduced litter survival indices; and reduced pup weights and pup survival indices of all litter intervals. At ophthalmoscopic evaluation, no effect of treatment at a dietary concentration of 6 ppm was seen in P_1 females before sacrifice, but for the F_1 animals, manifestations of ocular disease were noted both at initiation of the pre-mating period and at termination; ocular lesions were also seen with increased incidence among the F_1 animals during the weekly physical evaluations. No adverse effect of treatment at 6 ppm was evident from reproductive indices in either parental generation.

Gross postmortem evaluations of the P_1 and F_1 parental animals and selected F_{1b} and F_{2b} offspring after weaning revealed no adverse effect of treatment. External examination of weaned pups of treated females for each litter interval of each generation revealed no adverse effect of treatment.

Microscopic evaluation of the primary and secondary sexual organs and pituitary gland for animals of the P_1 and F_1 parental generations at 6 ppm revealed no treatment-related histomorphological alterations. Ocular lesions noted microscopically in the F_1 parental rats at 6 ppm were not considered to be caused by treatment, but appeared to have resulted from ocular infections acquired at an early age.

The NOAEL for parental and reproductive toxicity was 2 ppm (equal to 0.17 mg/kg bw per day for animals of the parental generations) on the basis of reduced pup growth, clinical findings, survival and inhibition of brain cholinesterase activity at 4 ppm (equal to 0.34 mg/kg bw) (Schroeder, 1991).

(b) Developmental toxicity

Rats

Groups of 25 pregnant Crl:COBS CD(SD)BR rats were given technical-grade phorate (purity, 91.7%) at a dose of 0, 0.125, 0.25 or 0.5 mg/kg bw per day by gastric intubation on days 6 to 15 of gestation, and were sacrificed on day 20 of gestation. The fetuses were removed for gross, skeletal and visceral examination. The pregnancy rate was comparable in all groups. Mortality was observed only in the group receiving phorate at a dose of 0.5 mg/kg bw per day (seven dams died). At 0.5 mg/kg bw per day, fetuses had an increased frequency of enlarged heart. Clinical signs, body weight and food consumption of dams during gestation, the number of implantation sites, the number of resorptions, the number of dead fetuses, mean live litter size, average fetal weight, sex ratio, and gross, skeletal and visceral abnormalities of fetuses were not significantly different from those of the controls. The NOAEL was 0.25 mg/kg bw per day (Beliles & Weir, 1979).

A pilot study that complied with the principles of GLP and that was certified for QA was carried out to determine the dose of technical-grade phorate to be used in a subsequent

study of developmental toxicity. Groups of eight mated female rats were given phorate (purity, 92.1%) at a dose of 0 (vehicle only—corn oil), 0.25, 0.5, 0.7 or 0.9 mg/kg bw per day on days 6–15 of gestation. The presence of spermatozoa or a copulatory plug was considered to be evidence of mating and the day on which it occurred was considered to be day 0 of gestation. Doses of ≥0.05 mg/kg bw per day were lethal; there were no surviving rats in the groups receiving phorate at a dose of 0.5, 0.7 or 0.9 mg/kg bw per day after day 12 of gestation. Overt clinical signs of toxicity preceding death included: twitches, tremors, excessive salivation, exophthalmos, urine-stained abdominal fur, ataxia, decreased motor activity, chromodacryorrhea, yellow anal/vaginal substance, chromorrhinorrhea, hyperactivity, clonic convulsion, lacrimation, soft or liquid faeces, emaciation, cold to touch, gasping and body jerks, and decreased body weight and food intake. Gross examination of rats that died revealed enlarged and/or congested adrenal glands. On the basis of these data, doses of 0, 0.1, 0.3 and 0.4 mg/kg bw per day were recommended for use in the definitive study of teratology in rats (Lochry, 1990).

A study of teratology was conducted to evaluate the developmental toxicity or the potential developmental toxicity (embryo-fetal toxicity/teratogenicity) of technical-grade phorate in Crl:CD®BR VAF/Plus® (Sprague-Dawley) rats. Groups of female rats were given phorate (purity, 92.1%) at a dose of 0 (vehicle), 0.1, 0.2, 0.3 or 0.4 mg/kg bw per day by gavage in corn oil once daily on days 6 to 15 of presumed gestation. There were 25 rats in the groups receiving phorate at a dose of 0 (vehicle) or 0.4 mg/kg bw per day, and 24 rats in each of the other treated groups. Phorate was prepared at a concentration of 0 (vehicle), 0.02, 0.04, 0.06 or 0.08 mg/ml, respectively, in a volume of 5 ml/kg, adjusted daily on the basis of the individual body weights recorded immediately before intubation. The rats were examined daily during and after dosing for clinical observations of the effects of the test substance, abortions, premature deliveries and deaths. Body weights and food consumption were recorded on day 0 of presumed gestation and daily during and after dosing. Rats that were found dead were necropsied on the day that death occurred. On day 20 of presumed gestation, the rats were sacrificed by carbon dioxide asphyxiation, and the abdomen of each rat was opened and examined for pregnancy, number and placement of implantations, early and late resorptions, live and dead fetuses and number of corpora lutea. Gravid uterine weights were recorded. Fetuses were subsequently examined for gross external, soft tissue and skeletal alterations.

Six rats receiving phorate at a dose of 0.4 mg/kg bw per day died after five to ten doses had been given (days 11–16 of presumed gestation). Of these, one rat was found dead on day 15 of presumed gestation and was not pregnant. All other rats were found dead on days 11, 15 or 16 of gestation and were pregnant. Each of these deaths were considered to be caused by administration of the test substance because of observations associated with the test substance (clinical observations, decreased body-weight gains and/or body-weight loss, decreased food consumption and necropsy lesions), and because eight out of eight rats in the groups receiving phorate at adose of 0.5, 0.7 or 0.9 mg/kg bw per day died in the pilot study.

Significantly increased numbers of rats at 0.4 mg/kg bw per day had tremors, chromodacryorrhea, urine-stained abdominal fur, decreased motor activity, chromorrhinorrhea, excess salivation, impaired righting reflex, a red substance around the nose and laboured breathing, compared with numbers in the control group. Increased numbers of rats in this group (0.4 mg/kg bw per day) had a red vaginal substance, red or tan oral substance and hunched posture, compared with numbers in the control group.

At necropsy, significantly increased numbers of rats at 0.4 mg/kg bw per day had urine-stained abdominal fur, chromodacryorrhea, a red, yellow or tan substance present around the eyes, nose and/or mouth and large adrenal glands, compared with numbers in the control group. Increased numbers of rats at 0.4 mg/kg bw per day also had a red or yellow substance present around the anal-vaginal area, as compared with numbers in the control group.

Administration of phorate at a dose of 0.4 mg/kg bw per day to the dams caused significant body weight loss, significant decreases in average maternal body-weight gains for the entire period of dosing and significantly reduced maternal body weights from day 12 of gestation until day 20 of gestation. Maternal body weight corrected for the gravid uterine weight was also significantly reduced for this group.

Significant decreases in food consumption occurred for the entire period of dosing and persisted after dosing, when compared with values for the control group. Fetal body weights were significantly decreased at 0.4 mg/kg bw per day. No other caesarean-delivery parameter was affected by administration of the test substance at doses as high as 0.4 mg/kg bw per day to the dams.

There were significant increases in the fetal and/or litter incidences of variations in skeletal ossification at 0.4 mg/kg bw per day. These variations were reversible delays in ossification of the sternum and pelvis and were expected observations that are related to the significantly decreased fetal body weights in this group. No malformations or variations revealed by gross external or soft tissue examination of the fetuses and no skeletal malformations were attributed to administration of the test substance.

The NOAEL for maternal and developmental toxicity was 0.3 mg/kg bw per day on the basis of mortality, clinical signs of toxicity, significantly decreased body weights and food consumption in the dams, and decreased fetal body weights and potentially reversible delays in skeletal ossification at 0.4 mg/kg bw per day. No fetal malformations were produced even at a (lethal) dose of 0.4 mg/kg bw per day, the highest dose tested. Therefore, phorate is not a developmental toxicant (Lochry, 1990).

Rabbits

A range-finding study of teratology was undertaken to provide preliminary data on the maternal toxicity, embryotoxicity and/or fetoxicity of phorate in the pregnant rabbit. Groups of five mated New Zealand white rabbits were given phorate (purity, 92.1%; dissolved in corn oil) at a dose of 0, 0.3, 0.6, 0.9, 1.2 or 1.5 mg/kg bw per day by gastric intubation on days 6–18 of gestation.

The incidences of mortality in the six groups were 0/5 (control) 1/5 (0.3 mg/kg bw per day), 1/5 (0.6 mg/kg bw per day), 1/5 (0.9 mg/kg bw per day), 2/5 (1.2 mg/kg bw per day) and 4/5 (1.5 mg/kg bw per day). Only one female at the highest dose survived to scheduled sacrifice. Food intake was generally decreased in all the treated groups, although no clear dose–response relationship was seen. Increased numbers of resorptions and postimplantation losses were observed at ≥0.6 mg/kg bw per day. Decreased mean fetal body weights and shorter crown–rump lengths were noted at 1.2 mg/kg bw per day. External examination of the fetuses showed no treatment-related malformations or alterations.

The only significant effects observed at the LOAEL of 0.3 mg/kg bw per day were a single maternal death and depressed food intake (Schroeder & Daly, 1986).

In the subsequent study of teratology in rabbits, groups of 20 mated female New Zealand white rabbits were given technical-grade phorate (purity, 92.1%; mixed with corn oil) at a dose of 0.15, 0.5, 0.9 or 1.2 mg/kg bw per day (derived from the range-finding study described above; Schroeder & Daly, 1986) by gastric intubation on days 6–18 of gestation. A control group of 20 mated females received vehicle (corn oil) only at a comparable volume as treated animals (2 ml/kg bw per day) on days 6–18 of gestation.

During gestation, females were observed twice daily for overt toxicity and morbidity or mortality. Animals were given a detailed physical examination and weighed on days 0, 6, 9, 12, 15, 18, 24 and 30 of gestation. Additionally, food consumption was recorded at several daily intervals during gestation. Animals experiencing abortion or delivering prematurely before scheduled sacrifice were killed on the day such events were seen. Surviving females were sacrificed on day 30 of gestation and given a gross postmortem examination. The ovaries and uterus were removed intact and weighed. The number of corpora lutea were recorded for each ovary and uterine implantations were identified as live, dead or resorbed fetuses. Fetuses recovered at this time were weighed, sexed (internal inspection of the gonads at visceral evaluation) and evaluated for external, visceral (microdissection procedure) and skeletal (Alizarin red S stained specimens) malformations or variations.

No treatment-related mortality was seen at a dose of 0.15 mg/kg bw per day. Mortality rates at 0.5, 0.9 and 1.2 mg/kg bw per day were 5.0% (1/20), 10.0% (2/20) and 40.0% (8/20), respectively; the mortality seen at these doses was considered to be treatment-related.

At doses of 0.15, 0.5 and 0.9 mg/kg bw per day, no adverse effects of treatment were evident from data on maternal weight, food consumption, physical observation or uterine implantation. Thus, these doses were not considered to be fetotoxic or teratogenic.

At the highest dose of 1.2 mg/kg bw per day, mean body weight on day 18 of gestation was significantly ($p < 0.05$) lower than that of the controls. During days 15–18 and 6–18 of gestation, this group experienced decreases in mean body weight and these data differed statistically ($p < 0.01$) from those for the control group, which experienced a slight gain in mean body weight over these same intervals. Food consumption at this dose was lower than that of the controls on days 15, 18 and 19 of gestation, but this was statistically significant ($p < 0.05$) only at day 18. During the physical observations, it was noted that several females receiving the highest dose had staining of the skin/fur in the ano-genital area during the treatment period; a similar observation was made at gross postmortem examination for several females that died during the treatment period. In addition to eight deaths that occurred before necropsy, one female aborted and another delivered prematurely. For the ten pregnancies evaluated at day 30 in this group receiving the highest dose, no adverse effects of treatment were evident from data on uterine implantation, fetal weights or fetal sex distribution. The only external malformation seen in the fetuses whose mothers received the highest dose was open eye, seen in all three fetuses from a single litter; these same fetuses when evaluated for skeletal malformation had curved scapulae. In this group, the female whose litter contained the fetuses with open-eye defects and curved scapulae experienced considerable toxicity as evidenced by a marked body-weight loss (778 g) during treatment and reductions in food consumption at days 15 and 18 of gestation. In the published literature, ocular malformations in rabbit fetuses have been identified as defects associated with maternal toxicity. Evaluation of the remaining 83 fetuses (nine litters) at the highest dose did not reveal an increase in malformations.

Phorate was not embryotoxic, fetotoxic or teratogenic at doses up to and including 1.2 mg/kg bw per day, a dose which produced severe maternal toxicity.

The NOAEL for maternal toxicity was 0.15 mg/kg bw per day on the basis of mortality observed at 0.5 mg/kg bw per day. The NOAEL for developmental toxicity was 1.2 mg/kg bw per day, the highest dose tested (Schroeder & Daly, 1987).

2.6 Special studies

(a) Neurotoxicity

(i) Single exposure

In a study of acute neurotoxicity, which complied with the principles of GLP and that was certified for QA, groups of 20 male and 20 female Sprague-Dawley CD® rats were given a single oral dose of technical-grade phorate (purity, 91.8%) at 0.25, 0.50, or 1.0 mg/kg bw (dose volume, 5 ml/kg bw) by gastric intubation. A control group of 20 animals of each sex received vehicle only (corn oil) at the same volume as the treated animals. Analysis of the dosing suspension for the group receiving the lowest dose (0.25 mg/kg bw) indicated that the correct concentration had not been prepared and administered. Therefore an additional group of 20 rats of each sex received a single oral dose of phorate at 0.25 mg/kg bw by gavage. Because animals in this additional group were dosed at a different time than those in the groups receiving the intermediate or highest doses, an additional control group of 20 animals of each sex) was included. An additional ten females were given phorate at a dose of 1.0 mg/kg bw before the initiation of the study in order to assess survival.

Physical observations, measurements of body weight and food consumption, and neurobehavioural evaluations (motor activity and functional observational battery) were performed on all animals pretest and at selected intervals during the treatment period. Blood samples were obtained from ten animals of each sex per group at the time of peak effect (approximately 4–5 h after dosing) for measurements of plasma cholinesterase and erythrocyte acetylcholinesterase activities. Immediately after blood collection, these animals were sacrificed and brains were obtained for the measurement of acetylcholinesterase activity. Blood samples for the measurement of plasma cholinesterase and erythrocyte acetylcholinesterase activities were collected from the remaining ten animals of each sex per group at 7 and 14 days after treatment. All animals were sacrificed at 14 days after treatment and a complete macroscopic examination was performed. Brains were obtained from five animals of each sex per group immediately after blood collection for the measurement of acetylcholinesterase activity. The remaining five animals of each sex per group, designated for neuropathology, were anaesthetized with an intraperitoneal injection of sodium pentobarbital and transcardially perfused with phosphate-buffered saline followed by 1% glutaraldehyde and 4% paraformaldehyde in the same buffer for histopathological evaluation of selected tissues of the central and peripheral nervous systems.

No mortalities were observed. Clinical findings noted during daily cage-side observations and weekly physical examinations were of the type commonly found in laboratory rats and were not considered to be treatment-related.

Mean body weights and body-weight gains for males and females in the treated groups (0.25, 0.5 or 1.0 mg/kg bw) were comparable to those for the respective controls throughout the study.

There were no treatment-related effects on food consumption for males or females in any treatment group compared with the respective control groups.

There were no statistically significant changes in mean motor activity on days 1, 8 and 15 for any treated group (0.25, 0.5 or 1.0 mg/kg bw) compared with the respective control groups.

Findings typical of those expected with cholinesterase inhibitors were made during the functional observational battery evaluations on day 1 in males and females at 0.5 and 1.0 mg/kg bw. Observations consisted of miosis for two males and two females at 0.5 mg/kg bw, and two males and five females at 1.0 mg/kg bw, compared with no males and one female in the control group. Additionally, at the highest dose, tremors were noted in one female, and tremors, fasciculations, slightly impaired locomotion, and splayed/dragging hindlimbs were noted in another. Recovery was evident during the 2-week observation period after dosing as the results of functional observational battery evaluations on days 8 and 15 were normal for all treatment groups.

Statistically significant inhibition of plasma cholinesterase activity and erythrocyte and brain acetylcholinesterase activities was observed on the day of dosing for males and females at 1.0 mg/kg bw when compared with controls. Plasma cholinesterase activity was inhibited by 27.5% and 67.7%; erythrocyte acetylcholinesterase activity was inhibited by 21.4% and 65.1%; and brain acetylcholinesterase activity was inhibited by 14% and 65.2%, for males and females, respectively, when compared with controls (Table 10).

Brain acetylcholinesterase activity was statistically significantly reduced on the day of dosing for males at 0.5 mg/kg bw when compared with controls. However, based on the small magnitude of this decrease (6.1%) and the lack of a similar statistically or biologically significant decrease in brain acetylcholinesterase activity at 0.5 mg/kg bw for females (females being more sensitive to phorate-induced inhibition of cholinesterase activity), this slight decrease in brain acetylcholinesterase activity for males at 0.5 mg/kg bw was not considered to be biologically significant.

Table 10. Inhibition of mean cholinesterase activity relative to controls in rats given a single oral dose of phorate

Dose (mg/kg bw per day)	Inhibition (%) of mean cholinesterase activity							
	Plasma			Erythrocyte			Brain	
	Day							
	1	8	15	1	8	15	1	15
Males								
0.25	1.7	6.3	4.5	−7.6[a]	5.8	4.2	2.3	1.8
0.5	−1.2[a]	−9.0[a]	−8.2[a]	10.5	6.2	−2.8[a]	6.1*	−0.7[a]
1.0	27.5**	0.7	0.6	21.4**	10.2	22.1	14.0**	7.2
Females								
0.25	−3.5[a]	5.7	−2.2[a]	−17.5[a]	−1.1[a]	−16.0[a]	0.5	−2.3[a]
0.5	18.7	−0.5[a]	0.0	5.1	17.1	2.3	3.1	−3.1
1.0	67.7**	4.0	7.5	65.1**	27.1	15.1	65.2**	9.3

From Mandella (1998)

* Significantly different from mean for controls; $p < 0.05$

** Significantly different from mean for controls; $p < 0.01$

[a] Apparent enhancement of cholinesterase activity

Recovery was evident in plasma cholinesterase activity and erythrocyte acetylcholinesterase activity by 8 days after dosing and in brain acetylcholinesterase activity by 14 days after dosing.

There were no macroscopic or microscopic findings at any dose that were related to treatment with phorate.

The NOAEL for inhibition of plasma cholinesterase and erythrocyte and brain acetylcholinesterase was 0.5 mg/kg bw on the basis of statistically significant reductions in plasma cholinesterase activity and erythrocyte and brain acetylcholinesterase activities on day 1 for males and females at 1.0 mg/kg bw, the highest dose tested. The NOAEL for neurobehavioural effects was 0.5 mg/kg bw on the basis of tremors in two females, and fasciculations, slightly impaired locomotion and splayed/dragging hindlimbs in one female at 1.0 mg/kg bw, the highest dose tested. The NOAEL for motor activity effects and neuropathology findings was 1.0 mg/kg bw, the highest dose tested. The overall NOAEL was 0.25 mg/kg bw on the basis of changes observed in the functional observational battery (miosis) in two males and two females at 0.5 mg/kg bw, the next highest dose tested (Mandella, 1998).

(b) Repeated exposure

In a 13-week study that complied with the principles of GLP and that was certified for QA, groups of 20 male and 20 female Sprague-Dawley CD® rats were given diets containing technical-grade phorate (purity, 91.8%) at a concentration of 0.5, 1.0, or 7.0 ppm for males and 0.5, 1.0, or 4.0 ppm for females. A control group of 20 animals of each sex received standard laboratory diet mixed with the carrier, acetone.

The dietary concentrations tested in the 13-week study were selected based on results from a pilot range-finding study of 21 days duration (Mandella, 1999b), which showed a 33% and a 87.9% decrease in erythrocyte acetylcholinesterase activity for males at 2.0 and 7.0 ppm, respectively, and a 39.4% and 100% decrease in erythrocyte acetylcholinesterase activity for females at 2.0 and 5.0 ppm, respectively. Additionally, brain acetylcholinesterase activity was decreased by 43.6% for males at 7.0 ppm and by 6.5% and 80.8% for females at 2.0 and 5.0 ppm, respectively. No effects on erythrocyte or brain acetylcholinesterase activity were noted for males and females at 1.0 ppm, the lowest concentrations tested for the respective sexes.

Mean daily intakes of phorate in the definitive study are summarized in Table 11.

Table 11. Mean daily substance intake in rats fed diets containing phorate for 13 weeks

Dietary concentration (ppm)	Mean daily intake (mg/kg bw)	
	Males	Females
0.5	0.037	0.041
1.0	0.074	0.081
4.0	—	0.334
7.0	0.538	—

From Mandella (1999a)

Physical observations and measurements of body weight and food consumption were performed for all animals before the start of the study and at selected intervals during the treatment period. Ophthalmoscopic examinations were conducted for all animals before the start of the study and for 10 animals of each sex per group (designated for evaluation of neurobehaviour) at termination. Motor activity and a functional observational battery (FOB) of behavioural tests were performed for 10 animals of each sex per group (designated for evaluation of neurobehaviour) before the start of the study and during weeks 4, 8 and 13 of treatment. Blood samples for the measurement of plasma cholinesterase and erythrocyte acetylcholinesterase activities were obtained from 10 animals of each sex per group (designated for evaluation of cholinesterase activity) during weeks 4, 8 and 13 of treatment and from five animals of each sex per group (designated for evaluation of neurobehaviour) at termination of treatment. At study termination, the brains from up to 15 animals of each sex per group (five animals of each sex in the group designated for evaluation of neurobehaviour and up to 10 animals of each sex in the group designated for evaluation of cholinesterase activity) were removed for measurement of acetylcholinesterase activity. The remaining five animals of each sex per group, designated for neuropathology, were anaesthetized with an intraperitoneal injection of sodium pentobarbital and transcardially perfused with phosphate-buffered saline followed by 1% glutaraldehyde and 4% paraformaldehyde in the same buffer for histopathological evaluation of selected tissues of the central and peripheral nervous systems. Complete macroscopic examinations were performed on all animals at necropsy.

Analysis of dietary mixtures confirmed that they were homogeneous. Stability analyses conducted for this study and for the range-finding study demonstrated that the test substance was stable in the diet for at least 14 days at room temperature and for at least 7 days (low concentration) or 14 days (high concentration) when stored refrigerated or frozen. Analysis of dietary mixtures during the treatment period confirmed that diets containing phorate at the appropriate concentration were administered.

There were no mortalities during the study. Clinical findings noted during daily cage-side observations and weekly physical examinations were of the type commonly found in laboratory rats and were not considered to be related to administration of the test substance. No test substance-related ocular abnormalities were noted at termination.

There were no statistically significant differences in motor activity at week 4, 8 or 13 for males or females in any treated group when compared with that of the control animals. Functional observational battery evaluations during weeks 4, 8 or 13 did not indicate any neurobehavioural effects of the test substance. There were no findings indicative of a cholinergic effect. Miosis was noted at week 4 for one of ten females at 1.0 ppm. This finding was considered to be an incidental finding and not related to treatment because the finding was not dose-related (as miosis was not noted at any interval in females at 4.0 ppm), and because miosis was not observed in the affected animal at weeks 8 or 13 after additional treatment with the test substance. A slight, but statistically significant, decrease in hindlimb grip strength was noted at week 4 for females at 1.0 ppm, relative to the control value. This decrease was not considered to be treatment-related because: the finding was not dose-related as statistically significant decreases in hindlimb grip strength were not observed at any interval in females at 4.0 ppm; a statistically significant decrease in hindlimb grip strength was only observed in one of the two trials at week 4 for females in the group at 1.0 ppm; and statistically significant decreases in hindlimb grip strength were not observed for females at 1.0 ppm at week 8 or 13 after further treatment with the test substance.

No adverse treatment-related effects on body weight, overall (weeks 1 to 13) body-weight gain or food consumption were observed.

Plasma cholinesterase activity (for animals in the groups designated for evaluation of cholinesterase activity or neurobehaviour) was statistically significantly decreased, relative to values for the respective controls, for males at 7.0 ppm and for females at 4.0 ppm. The inhibition of activity for animals in the group designated for evaluation of cholinesterase activity at weeks 4, 8 and 13 was 30.9%, 47.6% and 40.3%, respectively, for males at 7.0 ppm and 60.2%, 67.6% and 65.5%, respectively, for females at 4.0 ppm. The inhibition of plasma cholinesterase activity for animals in the group designated for evaluation of neurobehaviour at termination was 44.0% for males at 7.0 ppm and 73.6% for females at 4.0 ppm.

Erythrocyte acetylcholinesterase activity (for animals in the groups designated for evaluation of cholinesterase activity or neurobehaviour), was statistically significantly decreased, relative to values for the respective controls, for males at 7.0 ppm and for females at 4.0 ppm. The inhibition for males at 7.0 ppm was 84.4%, 91.2% and 78.4% for the animals in the group designated for evaluation of cholinesterase activity in weeks 4, 8 and 13, respectively, and 72.2% for the animals in the group designated for evaluation of neurobehaviour at termination. The inhibition for females at 4.0 ppm was 80.6%, 96.4% and 75.5% for the animals in the group designated for evaluation of cholinesterase activity at weeks 4, 8 and 13, respectively, and 82.4% for animals in the group designated for evaluation of neurobehaviour at termination. Erythrocyte acetylcholinesterase activity was decreased by 22.9% in week 4 for females at 1.0 ppm. This decrease was not statistically different from that in controls and was therefore not considered to be treatment-related. Moreover, erythrocyte acetylcholinesterase activity was decreased by only 11.1% at week 8 for females designated for evaluation of cholinesterase activity and was comparable to controls at termination for females designated for evaluation of cholinesterase activity or neurobehaviour.

Brain acetylcholinesterase activity (animals designated for evaluation of cholinesterase activity or neurobehaviour) was statistically significantly decreased at termination, relative to values for the respective controls, for males at 7.0 ppm and for females at 4.0 ppm. The inhibition of activity was 55.0% and 45.4% for males in the groups designated for evaluation of cholinesterase activity or neurobehaviour, respectively, and 66.6% and 64.7% for females, in the groups designated for evaluation of cholinesterase activity or neurobehaviour, respectively (Table 12).

There were no macroscopic findings related to administration of the test substance. No neuropathological effects were noted upon microscopic examination of tissues for males at 7.0 ppm or females at 4.0 ppm.

The NOAEL for this study was 1 ppm (equal to 0.07 mg/kg bw per day) on the basis of reduced erythrocyte and brain cholinesterase activities at the next highest dose (7 ppm in males and 4 ppm in females) (Mandella, 1999a).

(c) Delayed neurotoxicity

In this study of demylenation, groups of six adult white Leghorn hens were fed diets containing phorate (purity not specified) at a concentration of 0 or 40 ppm, equivalent to a dose of 5 mg/kg bw per day, for 4 weeks. Tri-ortho-tolyl phosphate at a dietary concentra-

Table 12. Inhibition of mean cholinesterase activity relative to controls in rats fed diet containing phorate for 13 weeks

Dietary concentration (ppm)	Inhibition (%) of mean cholinesterase activity									
	Plasma				Erythrocyte				Brain	
	Week (s)									
	4	8	13	NT	4	8	13	NT	13	NT
Males										
0.5	−15.3°	0.5	−5.2°	9.8	6.0	5.4	5.0	−2.8°	0.9	1.9
1	−2.3[a]	10.2	8.5	9.9	19.2	1.7	1.2	6.6	1.0	−2.0[a]
7	30.9*	47.6**	40.3**	44.0**	84.4**	91.2**	78.4**	72.2**	55.0**	45.4*
Females										
0.5	4.3	7.0	13.8	−24.2[a]	12.7	15.8	2.5	15.2	0.1	−1.7[a]
1.0	−8.0[a]	−5.4[a]	−2.3a	14.3	22.9	11.1	−1.4a	−4.6a	3.4	−2.7a
4.0	60.2**	67.6**	65.5**	73.6	80.6**	96.4**	75.5**	82.4**	66.6**	64.7*

From Mandella (1999a)
* Significantly different from mean for controls; $p < 0.05$
** Significantly different from mean for controls; $p < 0.01$
[a] Apparent enhancement of cholinesterase activity
NT: neurotoxicity group of rats, evaluated at week 13

tion of 4000 ppm was used as the positive control. Each hen was anaesthetized and immediately perfused with buffered formalin; and sections of brain, lower thoracic cord and each sciatic nerve were prepared for microscopic examination. Tri-ortho-tolyl phosphate induced loss of myelin in the nerve tissue in each hen, but phorate had no adverse effects on nerve fibres or their myelin sheaths (Morici & Levinskas, 1965).

A study of acute delayed neurotoxicity was conducted to determine potential neurotoxic effects of technical-grade phorate in mature white Leghorn hens (aged 22–23 months). In the first phase of the study, the acute oral median lethal dose (LD_{50}) of phorate was determined by dosing hens with phorate dissolved in corn oil. The estimated LD_{50} was 14.2 mg/kg bw.

In the second phase of the study, 50 fasted hens each received a single dose of phorate at 14.2 mg/kg bw on day 0, 1 h after being given an intramuscular injection of atropine sulfate at a dose of 10 mg/kg bw. An additional 15 fasted, atropinized hens were given corn oil only and 15 hens that did not receive atropine were given tri-ortho-toyl phosphate at a dose of 500 mg/kg bw as a positive control. All surviving hens in all groups received the same doses 21 days later, except that the administered dose of atropine sulfate was changed to 30 mg/kg bw. All hens were observed daily for mortality, clinical signs and evidence for neurotoxicity. Body weights and food consumption were recorded every 3 days. All hens that died during the study and all hens that were killed at the end of the study at day 42 were subjected to gross necropsy. Those killed at the end of the study were perfused with 10% neutralized formalin; and brains, vertebral columns (with spinal cord in situ) and the entire right and left sciatic nerves were excised and fixed. Microscopic slides of neural tissue were prepared by taking a sagittal section of the entire brain (corpus striatum, cerebellum, pons), longitudinal and cross-sections of the cervical, thoracic and lumbrosacral levels of the spinal cord and both sagittal and longitudinal sections of the right and left sciatic nerves. Sections were stained with haematoxylin and eosin, and replicate sections were stained with luxol fast blue. Tissues from 10 hens in each group were examined microscopically.

Of the 50 hens that were treated with phorate, 27 died within 24 h after the first dose and 13 more died within 24 h after the second dose. Ten hens survived to the termination of the study. No hens in the vehicle control group died during the 42-day study. All 15 hens in the positive control group were killed in extremis on day 16 of the study after clinical signs of neuropathy, first observed on day 11, became progressively more severe. These signs included generalized weakness, ataxia and paralysis of the legs and wings.

Hens in the vehicle control group and hens treated with phorate had slight generalized weakness of the limbs, lasting about 2 h, shortly after each treatment of atropine sulfate; the reaction was slightly more severe hens treated with phorate and these animals also had slight to moderate ataxia for up to 2 h after treatment. However, no clinical signs of delayed neuropathy were observed in any hen in the vehicle control group or in the group receiving phorate. In comparison with those of the vehicle controls, the mean body-weight gains of hens treated with phorate were higher at days 0–21 and lower at days 21–42, while food consumption of the treated hens was lower at days 0–21 and higher at days 21–42. No gross adverse effects attributable to phorate were observed at necropsy.

Histopathological examination of the neural tissues from the hens in the positive control group revealed treatment-related lesions involving the brain, spinal cord and/or sciatic nerves in all 10 birds. Generally, mild to moderate axonal degeneration was observed in the brains of 4 out of 10 hens, in the spinal cords of 10 out of 10 hens and in the sciatic nerves of 7 out of 10 hens; Schwann cell hyperplasia was also observed in the sciatic nerves of 3 out of 10 hens. These lesions were compatible with a delayed neurotoxic response induced by tri-ortho-tolyl phosphate. Minimal to mild focal axonal degeneration of the sciatic nerves was noted in 3 out of 10 hens treated with phorate; no axonal degeneration was seen in hens in the vehicle control group. The axonal degeneration observed in hens treated with phorate was associated with interstitial infiltration of lymphoid cells, which was also observed in other treated hens and in hens in the vehicle control group. This syndrome, which was distinct from that observed in hens in the positive control group, was ascribed to lesions of a naturally occurring disease, Marek disease, and was considered not to be treatment-related.

Thus, phorate did not induce clinical or histopathological signs indicative of acute delayed neuropathology (Fletcher, 1984).

2.7 Studies with metabolites

(a) Single exposure

In study of acute oral toxicity, which complied with the principles of GLP and was certified for QA, groups of five male and five female Sprague-Dawley rats (Crl : CD(SD)BR strain) were given phorate sulfone (purity, 99.4%; a metabolite of phorate) at a dose of 40, 20, 10, 5, 2.5, 1.75 (males and females), 1.25 and 0.625 (females only) mg/kg bw in corn oil by oral gavage. The animals were fasted overnight (approximately 18 h) before dosing. The animals were observed daily for overt signs of toxicity during the 14-day test period. Body weights were recorded on the day of dosing (day 0), day 7 and at termination (day 14). Necropsies were performed on all decedents during the study and on all survivors at the end of the 14-day observation period.

The mortality observed is summarized in Table 13.

Table 13. Mortality in rats given a single dose of phorate sulfone by gavage

Dose (mg/kg bw)	Mortality (No. died/No. dosed)		
	Males	Females	Combined
40.0	5/5	5/5	10/10
20.0	5/5	5/5	10/10
10.0	5/5	5/5	10/10
5.0	5/5	5/5	10/10
2.5	0/5	5/5	5/10
1.75	0/5	—	0/5
1.25	—	3/5	3/5
0.625	—	0/5	0/5

From Fischer (1990a)

Table 14. Mortality in rats given a single dose of phorate sulfoxide by gavage

Dose (mg/kg bw)	Mortality (No. died/No. dosed)		
	Males	Females	Combined
20.0	5/5	5/5	10/10
10.0	5/5	5/5	10/10
5.0	5/5	5/5	10/10
2.5	2/5	5/5	7/10
1.875	—	0/5	0/5
1.25	0/5	0/5	0/10

From Fischer (1990b)

Overt signs of toxicity were observed at all doses except 0.625 mg/kg bw. Signs of toxicity included decreased activity, salivation, tremors, chromodacryorrhea, ataxia and twitching limbs. Mortality was observed at all doses except 1.75 and 0.625 mg/kg bw. Mortality generally occurred during the first 8 h after dosing. Body-weight gains in surviving rats were generally unaffected by administration of the test substance. Gross pathological changes observed in decedents included external evidence of salivation and lacrimation, blood around the nose, congested livers and haemorrhagic lungs. There were no gross lesions observed in surviving rats which could attributed to ingestion of the test substance.

Based on the mortality data, the oral LD_{50} of phorate sulfone was 3.5 mg/kg bw in male rats and 1.2 mg/kg bw in female rats (no ranges calculable). The LD_{50} for both sexes combined was 2.5 mg/kg bw (no range calculable) (Fischer, 1990a).

In a study of acute oral toxicity with the phorate metabolite phorate sulfoxide, groups of five male and five female Sprague-Dawley rats (Crl : CD(SD)BR strain) were given phorate sulfoxide (purity, 91.8%) at a dose of 1.25 (both sexes), 1.875, 2.5, 5, 10 or 20 (females only) mg/kg bw in corn oil by oral gavage. The animals were fasted overnight (approximately 18 h) before dosing. The animals were observed daily for overt signs of toxicity during the 14-day test period. Body weights were recorded on the day of dosing (day 0), day 7 and at termination (day 14). Necropsies were performed on all decedents during the study and on all survivors at the end of the 14-day observation period.

The mortality observed is summarized in Table 14.

Overt signs of toxicity were observed at all doses except 1.875 mg/kg bw. Signs of toxicity included decreased activity, salivation, tremors, chromodacryorrhea and piloerection. Mortality was observed at all doses except 1.875 and 1.25 mg/kg bw. Mortality generally occurred during the first 2 h after dosing. Body-weight gains in surviving rats were generally unaffected by administration of the test substance. Gross pathological changes observed in decedents included external evidence of salivation and lacrimation, blood around the nose, chromodacryorrhea, congested kidneys and haemorrhagic lungs. There were no gross lesions observed in surviving rats which could be attributed to ingestion of the test substance.

Based on the data on mortality, the oral LD_{50} of phorate sulfoxide was 2.6 mg/kg bw in male rats and 2.2 mg/kg bw in female rats (no ranges calculable). The LD_{50} for both sexes combined was 2.4 mg/kg bw (no range calculable) (Fischer, 1990b).

(b) Repeated exposure

A 13-week study was conducted to evaluate the toxicity of phorate sulfone (purity, 92%; also containing about 6% unchanged phorate and 2% phorate sulfoxide) in groups of 30 male and 30 female Charles River CD® strain albino rats given diets containing phorate sulfone at a concentration of 0 (50 animals of each sex), 0.32, 0.80 or 2.0 ppm.

No mortality was observed in the study. There were no treatment-related changes in appearance or behaviour. Body-weight gain and increased food consumption were seen in males at 0.8 or 2 ppm, while no differences were observed in females when compared with control animals.

The mean daily intake of phorate sulfone is summarized in Table 15.

Plasma, erythrocyte and brain cholinesterase activities were measured. Plasma cholinesterase activity was reduced by 23–27% in males at 2 ppm at weeks 1, 3 and 5 and by 25–72% in females at 2 ppm at all time-points. Plasma cholinesterase activity was also inhibited (39%) in females at 0.8 ppm at weeks 1 and 3. Erythrocyte acetylcholinesterase activity was reduced by ≥20% in both males and females at most intervals. Brain acetylcholinesterase activity was inhibited (>20%) in females at 2 ppm at weeks 3, 5 and 8.

No treatment-related effects were observed in erythrocyte volume fraction, haemoglobin, total and differential leukocyte counts and kidney and liver weights. No adverse gross and microscopic alterations were recorded.

The NOAEL was 0.80 ppm, equal to 0.08 mg/kg bw per day, on the basis of inhibition of erythrocyte and brain cholinesterase activities (Hutchinson et al., 1968a).

Table 15. Mean daily substance intake in rats fed diets containing phorate sulfone for 13 weeks

Dietary concentration (ppm)	Mean daily intake (mg/kg bw)	
	Males	Females
0.32	0.032	0.043
0.80	0.079	0.107
2.0	0.196	0.259

From Hutchinson et al. (1968a)

Table 16. Mean daily substance intake in rats fed diets containing phorate sulfoxide for 13 weeks

Dietary concentration (ppm)	Mean daily intake (mg/kg bw)	
	Males	Females
0.32	0.024	0.028
0.80	0.060	0.068
2.0	0.149	0.172

From Hutchinson et al. (1968b)

In a 13-week study of toxicity, groups of 35 male and 35 female Charles River CD® strain albino rats were given diets containing phorate sulfoxide (purity, 93%; also containing about 2% unchanged phorate and 5% phorate sulfone) at a concentration of 0 (50 animals of each sex), 0.32, 0.80 or 2.0 ppm.

Two animals died during the study; a male in the control group at day 50 and a male from the group receiving phorate at 2 ppm at day 75. There were no treatment-related changes in appearance or behaviour. No significant differences in body-weight gain and food consumption were observed.

Mean daily intakes of phorate sulfoxide are summarized in Table 16.

Plasma, erythrocyte and brain cholinesterase activity was measured at weeks 1, 3, 5, 8 and 12. Plasma cholinesterase activity was significantly reduced in males at 2 ppm at week 3 (84%) and in females at 2 ppm at weeks 1 (44%), 3 (50%) and 12 (51%); plasma cholinesterase was significantly inhibited (62%) in females at 0.8 ppm at week 3. Erythrocyte acetylcholinesterase activity was significantly reduced in males at 2 ppm at weeks 1 (23%), 3 (30%) and 12 (26%) and in females at 2 ppm at weeks 3 (46%), 5 (45%), 8 (60%) and 12 (48%); Brain acetylcholinesterase activity was significantly inhibited in males at 2 ppm at weeks 3 (21%) and 8 (11%) and in females at all intervals (18–29%); brain acetylcholinesterase activity was also significantly reduced in males at 0.8 ppm at week 3 (16%).

No treatment-related effects were observed in erythrocyte volume fraction, haemoglobin, total and differential leukocyte counts or kidney and liver weights. No adverse gross and microscopic alterations were recorded.

The NOAEL was 0.80 ppm, equal to 0.060 mg/kg bw per day on the basis of brain cholinesterase inhibition (Hutchinson et al., 1968b).

3. Observations in humans

In a pesticide formulation plant, cases of poisoning have been reported for two workers who were engaged in the formulation of Thimet. The symptoms of intoxication were dizziness, nausea, vomiting, constricted pupils, cardiac tachycardia, excessive salivation, respiratory distress, muscle fasciculations, and pin-point pupils. After treatment with atropine and/or 2-PAM (2-pyridine-aldoximemethiodide), both men recovered. Concentrations of phorate in the air in the plant ranged from 0.07 to 14.6 µg/l. No cholinesterase measurements were reported (Young et al., 1979; WHO, 1988). In another incident, a formulator experienced neurological symptoms (not specified) after exposure to phorate while

cleaning a mixing tank. Plasma cholinesterase and erythrocyte acetylcholinesterase activities were reduced by 50% of base-line values and increased concentrations of diethyl phosphate in urine, a metabolite of phorate, were also observed (WHO, 1988). Forty male workers who were engaged in the formulation of phorate for 2 weeks developed toxic symptoms, including gastrointestinal effects, bradycardia, and neurological effects (headache, giddiness, fatigue). Dermal and ocular irritation also occurred. In 60% of the subjects, mean plasma cholinesterase activity was decreased by 55% at the end of the first week and by 71% at the end of the second week compared with pre-exposure activity. Within 10 days after cessation of exposure, cholinesterase activity had recovered to 70% of pre-exposure levels (Kashyap et al., 1984).

Comments

In rats treated orally with radiolabelled phorate, 77% of the administered dose was recovered in the urine within 24 h after dosing. Faecal excretion accounted for approximately 12% of the administered dose. Over the total duration of the study (192 h), essentially the entire administered dose was eliminated by excretion.

Phorate was highly toxic when administered orally, dermally or by inhalation. The oral LD_{50}s for rats were 3.7 mg/kg bw in males and 1.4 mg/kg bw in females. The dermal LD_{50}s for rats were 9.3 mg/kg bw in males and 3.9 mg/kg bw in females. The LC_{50}s for rats after an exposure of 1 h were 0.06 and 0.011 mg/l of air in males and females respectively. Studies of dermal and eye irritation and of dermal sensitization were not performed owing to the high acute toxicity of phorate by dermal contact.

The toxicological effects of phorate are associated with inhibition of acetylcholinesterase activity. Inhibition of acetylcholinesterase activity and clinical signs occurred at similar doses in rats, rabbits and dogs, while mice appeared to be somewhat less sensitive. The NOAELs for toxicologically significant inhibition of brain acetylcholinesterase activity were 0.05–0.07 mg/kg bw per day in 13-week and 2-year studies in rats and in 1-year studies in dogs. The NOAELs for clinical signs were generally higher. The Meeting noted that the dose–response curve for acetylcholinesterase inhibition is steep.

In an 18-month study in mice and in a 24-month study in rats, phorate did not increase the incidence of tumours or cause any non-neoplastic effects other than clinical signs secondary to inhibition of acetylcholinesterase activity.

Phorate was tested for genotoxicity in vitro and in vivo in an adequate battery of assays. In view of the lack of genotoxicity in vitro and in vivo, and on the basis of the results of studies of carcinogenicity in rodents, the Meeting concluded that phorate is not likely to pose a carcinogenic risk to humans.

In a multigeneration study of reproductive toxicity in mice, the NOAEL was 1.5 ppm (equal to 0.30 mg/kg bw per day) on the basis of slightly reduced lactation indices in four out of the six litters at 3 ppm (equal to 0.60 mg/kg bw per day).

In a two-generation study of reproductive toxicity in rats, phorate showed effects on pup growth and mortality at maternally toxic doses. The NOAEL was 2 ppm (equal to 0.17 mg/kg bw per day) on the basis of decreased brain acetylcholinesterase activity,

decreased parental and pup body weights and decreased pup survival at 4 ppm (equal to 0.35 mg/kg bw per day).

In a study of developmental toxicity in rats, the NOAELs for maternal and developmental toxicity with phorate were 0.3 mg/kg bw per day on the basis of mortality, cholinergic clinical signs of toxicity, significantly decreased body weights and food consumption in the dams, decreased fetal body weights and delays in skeletal ossification at 0.4 mg/kg bw per day. No fetal malformations were produced, even at the lethal dose of 0.4 mg/kg bw per day, the highest dose tested. Therefore, the Meeting concluded that phorate is not a teratogen in rats.

Phorate was not embryotoxic, fetotoxic or teratogenic in rabbits at doses of up to and including 1.2 mg/kg bw per day, a dose that produced severe maternal toxicity. The NOAEL for maternal toxicity with phorate was 0.15 mg/kg bw per day on the basis of mortality observed at 0.5 mg/kg bw per day. The NOAEL for developmental toxicity was 1.2 mg/kg bw per day, the highest dose tested.

The Meeting concluded that the existing database on phorate was adequate to characterize the potential hazards to fetuses, infants and children.

In a study of acute neurotoxicity in rats treated by gavage, phorate at a dose of 1 mg/kg bw caused miosis in 2 out of 20 males and 5 out of 20 females, tremors in 2 out of 20 females, fasciculations, slightly impaired locomotion and splayed or dragging hindlimbs in one female, and significant inhibition of brain and erythrocyte acetylcholinesterase activity in females (65%), but not in males (14–21%). No histopathological signs were observed. At 0.5 mg/kg bw, miosis was observed in 2 out of 20 males and 2 out of 20 females. Although miosis was observed in a small number of animals (and in 1 out of 20 controls) in the absence of inhibition of erythrocyte and brain acetylcholinesterase activity, it could not be dismissed as a compound-related effect. The NOAEL was 0.25 mg/kg bw on the basis of miosis.

Phorate did not cause acute delayed neurotoxicity in hens. Although measurements of neuropathy target esterase were not carried out, the Meeting noted that the dose used (approximately equal to the LD_{50}) was sufficiently high to indicate that dietary exposure to phorate would not cause delayed polyneuropathy.

The toxicity of the mammalian and plant metabolites of phorate, phorate sulfone and phorate sulfoxide, was similar to that of the parent compound. In rats, the oral LD_{50}s for these metabolites were 1.2–3.5 and 2.2–2.6 mg/kg bw, respectively. The NOAELs for inhibition of brain acetylcholinesterase activity were 0.80 ppm (equal to 0.08 and 0.06 mg/kg bw per day) for phorate sulfone and sulfoxide respectively in 90-day studies in rats.

Several cases of occupational and non-occupational poisoning in humans have been reported. The subjects showed typical cholinergic symptoms, including gastrointestinal effects, bradycardia and neurological effects (headache, giddiness, fatigue). Dermal and ocular irritation were also observed.

Toxicological evaluation

An ADI of 0–0.0007 mg/kg bw was established on the basis of a overall NOAEL of 0.07 mg/kg bw per day for inhibition of brain acetylcholinesterase activity in rats and dogs, and a safety factor of 100. This ADI includes the phorate metabolites, phorate sulfone and phorate sulfoxide.

An acute reference dose (ARfD) of 0.003 mg/kg bw was also established based on the NOAEL of 0.25 mg/kg bw for miosis in the study with single doses in rats. Although inhibition of acetylcholinesterase activity is a C_{max}-dependent phenomenon, a safety factor of 100 was used in view of the steep dose–response curve and the slow recovery of brain acetylcholinesterase activity because of irreversibility of its inhibition. This ARfD includes phorate sulfone and phorate sulfoxide.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month study of toxicity and carcinogenicity[a]	Toxicity	3 ppm, equivalent to 0.45 mg/kg bw per day	6 ppm, equivalent to 0.90 mg/kg bw per day
		Carcinogenicity	6 ppm, equal to 0.90 mg/kg bw per day[d]	—
	Multigeneration study of reproductive toxicity[a]	Parental and offspring toxicity	1.5 ppm, equal to 0.30 mg/kg bw per day	3 ppm, equal to 0.60 mg/kg bw per day
Rat	2-year study of toxicity and carcinogenicity[a]	Toxicity	1 ppm, equal to 0.05 mg/kg bw per day	3 ppm, equal to 0.16 mg/kg bw per day
		Carcinogenicity	6 ppm, equal to 0.32 mg/kg bw per day[c,d]	—
	Multigeneration reproductive toxicity[a]	Parental and offspring toxicity	2 ppm, equal to 0.17 mg/kg bw per day	4 ppm, equal to 0.35 mg/kg bw per day
	Developmental toxicity[a]	Embryo- and fetotoxicity and maternal toxicity	0.3 mg/kg bw per day	0.40 mg/kg bw per day
	Single-dose study[c]	Miosis	0.25 mg/kg bw	0.50 mg/kg bw per day
	13-week study of neurotoxicity[a]	Neurotoxicity	0.07 mg/kg bw per day	0.3 mg/kg bw per day
Rabbit	Developmental toxicity[a]	Maternal toxicity	0.15 mg/kg bw per day	0.50 mg/kg bw per day
		Embryo- and fetotoxicity[a]	1.2 mg/kg bw per day[d]	—
Dog	1-year study of toxicity[b]	Toxicity	0.05 mg/kg bw per day	0.25 mg/kg bw per day

[a] Diet
[b] Capsules
[c] Gavage
[d] Highest dose tested

Estimate of acceptable daily intake for humans

0–0.0007 mg/kg bw

Estimate of acute reference dose

0.003 mg/kg bw

Studies that would provide information useful for the continued evaluation of the compound

Further observation in humans

Summary of critical end-points for phorate

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of oral absorption	Rapid, approximately 90% within 24 h
Dermal absorption	Extensive based on acute toxicity
Distribution	Rapid and extensive
Potential for accumulation	None
Rate and extent of excretion	89% within 24 h; urinary excretion predominated (77%); faecal excretion (12%)
Metabolism in animals	Major pathway: cleavage of phosphorus–sulfur bond, methylation of the liberated thiol group and oxidation of the resulting divalent moiety to the sulfoxide and sulfone
Toxicologically significant compounds (plants, animals and the environment)	Parent, phorate sulfoxide and phorate sulfone
Acute toxicity	
Rat, LD_{50}, oral	3.7 mg/kg bw in males, 1.4 mg/kg bw in females
Rat, LD_{50}, dermal	9.3 mg/kg bw in males, 3.9 mg/kg bw in females
Rat, LC_{50}, inhalation	0.06 mg/l of air in males (1 h), 0.011 mg/l of air (1 h) in females
Rabbit, dermal irritation	Highly toxic by skin contact—could not be tested
Rabbit, ocular irritation	Highly toxic by eye contact—could not be tested
Dermal sensitization	Highly toxic by skin contact—could not be tested
Short-term studies of toxicity	
Target/critical effect	Brain and erythrocyte acetylcholinesterase activity, and miosis (rats)
Lowest relevant oral NOAEL	0.07 mg/kg bw per day
Lowest relevant dermal NOAEL	No data
Lowest relevant inhalation NOAEC	No data
Genotoxicity	Negative results in vivo and in vitro
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Inhibition of erythrocyte and brain cholinesterase activity
Lowest relevant NOAEL	0.07 mg/kg per day (rat)
Carcinogenicity	Not carcinogenic in mice and rats
Reproductive toxicity	
Reproduction target/critical effect	Reduced pup growth at maternally toxic dose
Lowest relevant reproductive NOAEL	2 ppm, equivalent to 0.17 mg/kg bw per day
Developmental target/critical effect	Decreased pup weights and delayed ossification at maternally toxic doses (rats)
Lowest relevant developmental NOAEL	0.3 mg/kg bw per day (rats)
Neurotoxicity/delayed neurotoxicity	
Single dose study of neurotoxicity	
Target/critical effect	Signs consistent with acetylcholinesterase inhibition; no neuropathological effects
Relevant NOAEL	0.25 mg/kg bw
Delayed neuropathy	No delayed neurotoxicity in hens
Medical data	Findings consistent with inhibition of acetylcholinesterase activity; no record of permanent sequelae

Summary	Value	Study	Safety factor
ADI	0–0.0007 mg/kg bw	Rats and dogs, short- and long-term studies, inhibition of brain acetylcholinesterase activity	100
ARfD	0–0.003 mg/kg bw	Rats, single-dose study, miosis	100

References

Allen, J.S. (1978) Mutagenicity testing of technical THIMET* phorate (Lot No. AG 59,673 drum number 1) in the Ames bacterial test. American Cyanamid Company, Princeton, NJ, USA: *AIR*, **6**, 124–136.

Beliles, R.P. & Weir, R.J. (1979) Teratology study in rats: THIMET® phorate (lot No. 70513-4844-2). Unpublished report No 20819 from Litton Bionetics, Inc., Kensington, MD, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Blinn, R.C. (1982) Personal communication to WHO by American Cynamid Co., Wayne, NJ, USA.

Bowman, J.S. & Casida, J.E. (1958) Further studies on the metabolism of Thimet by plants, insects and mammals. *J. Econ. Entomol.,* **51**, 838–843.

Fischer, J.E. (1990a) Oral LD_{50} study in albino rats with CL 18,161 technical (lot No. AC 5105-90B). Unpublished report No. A90-204, toxicology study T-0288, from American Cyanamid Company, Princeton, NJ. USA. Submitted to WHO by BASF Limburgerhog, Germany.

Fischer, J.E. (1990b) Oral LD_{50} study in albino rats with CL 18,177 technical (lot No. AC 4870-30B). Unpublished report no A90-205, toxicology study T-0289, from American Cyanamid Company, Princeton, NJ. USA. Submitted to WHO by BASF Limburgerhog, Germany.

Fletcher, D.W. (1984) 42-Day neurotoxicity study with phorate in mature Leghorn chickens. Unpublished report No. 83 DN 103 from Bio-Life Associates Ltd, Neillsville WI USA. Submitted to WHO by BASF Limburgerhog, Germany.

Gaines, T.B. (1969) Acute toxicity of pesticides. *Toxicol. Appl.Pharmacol.*, **14**, 515–534.

Hussain, M. (1987) Thimet® insecticide, phorate (CL 35,024) in the rat. Unpublished report No. PD-D Volume 27–59 from American Cyanamid Co., Princeton, NJ, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Hutchinson, E.B., Fegley, H.C., McNerney, J.M. & Levinskas, G.J. (1968a) Sulfone of THIMET® (lot No. 1060-67-3), systemic insecticide: 90-day repeated feeding to albino rats (CL 181,161). Unpublished report No. 68-66 from American Cyanamid Company (Central Medical Department; Environmental Health Laboratory), Princeton, NJ, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Hutchinson, E.B., Fegley, H.C., McNerney, J.M. & Levinskas, G.J. (1968b) Sulfoxide of THIMET® (lot No. 1060-88A), systemic insecticide: 90-day repeated feeding to albino rats (CL 181,161). Unpublished report No. 68-65 from American Cyanamid Company (Central Medical Department; Environmental Health Laboratory), Princeton, NJ, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Ivett, J.L. & Myhr, B.C. (1986) Second amended report. chromosomal aberrations in vivo in mammalian bone marrow cells on AC 35,024 (Lot No.AC 4870-110). Unpublished report No. 22202 from Litton Bionetics, Inc., Kensington, MD, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Kashyap, S.K., Jain, J.P. & Saiyed, H.N. (1984) Clinical effects of cholinesterase activity changes in workers exposed to phorate (Thimet). *J. Environ. Sci. Health*, **19**, 479–489.

Kay, J.H. & Calandra, J.C. (1961) Effects of Thimet phorate on cholinesterase activity in the dog. Unpublished report from Industrial Bio-Test Laboratories, Inc., Northbrook, IL, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Kinsler, S. (1990) Relative contribution of the cytochrome P450 and flavin containing monooxygenases to the microsomal oxidation of phorate following treatment of mice with phenobarbital, hydrocortisone, acetone, and piperonyl butoxide. *Pesticide Biochem. Physiol*., **37**, 174–181.

Lochry, E.A. (1990) An Oral developmental toxicity (embryo-fetal toxicity/teratogenicity) definitive study with AC 35,024 (lot No. AC 6479-145) in rats. Unpublished report No. 101-012 from Argus Research Laboratories, Inc., Horsham, PA, USA: Submitted to WHO by BASF Limburgerhog, Germany.

Mandella, R. (1998) An acute neurotoxicity study with AC 35024 (lot No. AC 9429-41) in the rat via oral garage administration. Unpublished report No. 97-4520 from Huntingdon Life Sciences, East Millstone, NJ, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Mandella, R. (1999a) 13-Week dietary neurotoxicity study with AC 35042 (lot No. AC 9429-41) in the rat. Unpublished report No. 98-4522 from Huntingdon Life Sciences, East Millstone, NJ, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Mandella, R. (1999b) 21-Day dietary range-finding study with AC 35042 (lot No. AC 9429-41) in the rat. Unpublished report No. 98-2593 from Huntingdon Life Sciences, East Millstone, NJ, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Manus, A.G., Goldsmith, L.A. & Craig, D.K. (1981a) 18-Month chronic toxicity and potential carcinogenicity study on phorate (lot No. W-70513-4844-2) in mice. Unpublished report No. 20820 from Litton Bionetics, Inc., Kensington, MD, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Manus, A.G., Goldsmith, L.A. & Craig, D.K. (1981b) 24-Month chronic toxicity and potential carcinogenicity on phorate (lot No. W-70513-4588-2), study in rats. Unpublished report No. 20821 from Litton Bionetics, Inc., Kensington, MD, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Miller, P. & Wu, D. (1990) Phorate (CL 35,024): absorption, disposition, elimination and metabolic fate of carbon-14 CL 35,024 in the female rat. Unpublished report No. RPT0043, PD-M Volume 27-59 from Xeno-Biotic Laboratories, Inc., USA. Submitted to WHO by American Cyanamid Co., Princeton, NJ, USA.

Morici, I.J. & Levinskas, G.J. (1965) THIMET (lot No. AC 841-56A), systemic insecticide: demyelination studies in white Leghorn hens. Unpublished report No. 65-107 from American Cyanamid Company, Central Medical Department, Princeton, NJ, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Morici, I.J., Ribelin, W.E. & Levinskas, G.J. (1965) THIMET® (lot No. 40515-1), systemic insecticide: successive generation studies with mice. Unpublished report No. 65-136 from American Cyanamid Company, Central Medical Department, Princeton, NJ, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Morowati, M. (1999) Inhalation toxicity studies of Thimet (phorate) in the male Swiss albino mouse, *Mus musculus*: II. Lung histopathology, pseudocholinesterase level and hematological studies. *Environ. Pollution*, **103**, 309–315.

Newell, G.W. & Dilley, J.V. (1978) Teratology and acute toxicology of selected chemical pesticides administered by inhalation. Unpublished report No. 68-02-1751, EPA-600/1-78-003, from Stanford Research Institute, Menlo Park, CA, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Piccirillo, V.J., Dauvin, E.M. & Shellenberger, T.E. (1987) 14-Day range-finding oral toxicity study in the dog with AC 35,024 (lot No.AC 4870-110). Unpublished report No. 85013 from Tegeris Laboratories, Inc., Temple Hills, MD, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Schroeder, R.E. (1991) A two-generation (two-litters) reproduction study with AC 35,024 (lot No. AC 6479-145) to rats. Unpublished report No. 88-3350 from Bio/Dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Schroeder, R.E. & Daly, I.W. (1986) A range-finding teratology study with phorate (Lot No.AC 4870-110) in rabbits. Unpublished report No. 86-3038 from Bio/Dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Schroeder, R.E. & Daly, I.W. (1987) A teratology study with phorate (Lot No.AC 4870-110) in rabbits. Unpublished report No. 86-3039 from Bio/Dynamics Inc., East Millstone, NJ, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Shellenberger, T.E. & Tegeris, A.S. (1987) One-year oral toxicity study in purebred beagle dogs with AC 35,024 (lot No.AC 4870-110). Unpublished report No. 85015 from Tegeris Laboratories, Inc., Laurel, MD, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Simmon, V.F., Mitchell, A.D. & Jorgenson, T.A. (1977) Evaluation of selected pesticides (lot No. M685) as chemical mutagens in vitro and in vivo studies. Unpublished report No. 68-01-2458, EPA-600/1-77-028, from Stanford Research Institute, Menlo Park, CA, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Thilagar, A. & Kumarop, V. (1985) Test for chemical induction of gene mutation at the *Hgrpt* locus in cultured Chinese hamster ovary (CHO) cells with and without metabolic activation with AC 35024 (lot No.AC 4870-110). Unpublished report No. 0007-2500 from Sitek Research Laboratories, Rockville, MD, USA. Submitted to WHO by American Cyanamid Co., Princeton, NJ, USA.

Trutter, J.A. (1990) 13-Week dietary toxicity study in albino mice with AC 35,024 (lot No. AC 6479-145). Unpublished report No. 362-291 from Hazleton Laboratories America Inc., Vienna, VA, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Tusing, T.W., Kundzin, O.E., Hanzal, R. & Howard, J. (1956a) Repeated oral administration—dogs—experimental insecticidal 3811, 92% (lot No. not specified). Unpublished report BASF RDI No. PA-425-002 data from Hazleton Laboratories, Falls Church, VA, USA. Submitted to WHO by BASF Limburgerhog, Germany.

Tusing, T.W., Paynter, M., Kundzin, O.E., Hanzal, R. & Howard, J. (1956b) Subacute feeding study—rats—experimental insecticide 3911, 92% (lot No. not specified). Unpublished report BASF RDI No. PA-425-002, data from Hazleton Laboratories, Falls Church, VA, USA. Submitted to WHO by BASF Limburgerhog, Germany.

World Health Organization (1988) Phorate (WHO data sheets on pesticides, No. 75). Geneva, Switzerland.

Young, R.J., Jung, F.P. & Ayur, H.E. (1979) Phorate intoxication at an insecticide formulation. *Am. Ind. Hyg. Assoc. J.*, **40**, 13–16.

PIRIMICARB

First draft prepared by
D.B. McGregor
Toxicity Evaluation Consultants, Aberdour, Scotland

Explanation

Pirimicarb is the ISO approved common name for 2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate. It is a selective aphicide that is used extensively on a broad range of crops, including vegetable, cereal and orchard crops. The mode of action of pirimicarb is by inhibition of acetylcholinesterase activity.

Pirimicarb was evaluated by the JMPR in 1976, 1978 and 1982 (Annex 1, references 26, 30 and 38); an acceptable daily intake (ADI) of 0–0.02 mg/kg bw was established in 1983. Pirimicarb was reviewed by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues (CCPR), using new data not previously reviewed and relevant data from previous evaluations.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution and excretion

Groups of two male and two female rats were given either [^{14}C]pyrimidinyl-labelled pirimicarb (radiochemical purity, >98%) or [^{14}C]carbamoyl-labelled pirimicarb (radiochemical purity, >98%) as a single oral dose at 1 mg/kg bw. At intervals of 6 h and 24 h after

dosing, one rat of each sex from each group was killed and rapidly frozen for whole body autoradiography. The excretion of radioactivity in urine, faeces and exhaled air was monitored for 24 h.

Excretion of both [^{14}C]pyrimidinyl-labelled and [^{14}C]carbamoyl-labelled pirimicarb was rapid, with >50% of the administered radiolabel being eliminated within 6 h after dosing (Table 1). In the case of [^{14}C]pyrimidinyl-labelled pirimicarb, most of the excreted radiolabel was in the urine. Urinary excretion of radioactivity over 24 h accounted for 83% and 89% of the administered dose for male and female rats, respectively. Faecal excretion accounted for >6% of the administered radiolabel and about 0.1% was recovered from exhaled air. In the case of [^{14}C]carbamoyl-labelled pirimicarb, however, the principal route of elimination was via exhaled air, with >66% of the dose recovered as [^{14}C]labelled carbon dioxide over 24 h for both sexes. Urinary and faecal excretion accounted for <18% and 3% of the administered dose, respectively. These findings are consistent with the extensive cleavage of the carbamate moiety and the separate fates of this and the substituted pyrimidine ring.

Whole-body autoradiography indicated that there was widespread systemic distribution of radioactivity from both labelled compounds.

Six hours after the administration of [^{14}C]pyrimidinyl-labelled pirimicarb, there was a high concentration of the radiolabel in the liver, showing a reticular pattern of distribution. High levels were also seen in the renal medulla, gastrointestinal mucosa and gastrointestinal tract contents. Moderate labelling was observed in the Harderian gland and lower levels occurred in the remaining tissues. In rats killed 24 h after dosing, lower concentrations of radiolabel were observed in all tissues, the highest concentration being found in the liver.

Six hours after the administration of [^{14}C]carbamoyl-labelled pirimicarb, high levels of labelling were present in the buccal cavity, gastrointestinal tract contents, the gastric mucosa and in the liver, which showed a more homogeneous distribution of radioactivity than seen in the liver of rats given [^{14}C]pyrimidinyl-labelled pirimicarb. In the female rat, the retina and lens were also prominently labelled. After 24 h, there was some labelling in the seminal vesicles.

Table 1. Recovery (%) of radiolabel over 6 h and 24 h after administration of ^{14}C-labelled pirimicarb as a single oral dose at 1 mg/kg bw

Sample	[^{14}C]pyrimidinyl-pirimicarb				[^{14}C]carbamoyl-pirimicarb			
	Male		Female		Male		Female	
	0–6 h	0–24 h	0–6 h	0–24 h	0–6 h	0–24 h	0–6 h	0–24 h
Urine	64.4	83.2	50.4	89.4	8.6	14.2	8.8	17.0
Faeces	<0.1	5.3	NS	3.3	<0.1	2.6	NS	1.1
Carbon dioxide	NA	0.1	NA	0.1	NA	66.6	NA	66.8
Cage wash	2.4	5.7	19.4	2.4	1.3	3.6	2.2	0.7
Total	66.8	94.3	69.8	95.2	9.9	87.0	11.0	85.6

From Brown (1997a)
NA, not applicable; NS, no sample

There did not appear to be any pronounced sex difference in either the excretion profiles or in tissue distribution for either [^{14}C]pyrimidinyl- or [^{14}C]carbamoyl-labelled pirimicarb (Brown, 1997a).

The tissue distribution and excretion of pirimicarb-associated radioactivity was studied in groups of five male and five female Alpk:AP$_f$SD rats after oral administration of [^{14}C]pyrimidinyl-labelled pirimicarb (radiochemical purity, >99%) at a dose of 1 mg/kg bw in corn oil. The rats were housed individually in metabolism cages from which urine and faeces were collected daily. The rats were killed 4 days after dosing, when representative samples of tissues were removed and analysed for residual radioactivity by liquid scintillation counting. The results are presented in Tables 2 and 3.

Pirimicarb was extensively absorbed. Over 4 days, males excreted a mean of 92% of the administered dose, with 79% being eliminated in urine and 11% in the faeces. Over the same period, females excreted >90% of the administered dose, with 82% in the urine and 7% in the faeces. The rate of excretion was rapid with >80% of the administered dose being excreted within 24 h after dosing in both sexes. Excretion was essentially complete within 4 days. No sex difference was apparent in either the routes or rates of excretion.

Table 2. Mean recovery (%) of radiolabel 4 days after administration of [^{14}C]pyrimidinyl-labelled pirimicarb at a single oral dose of 1 mg/kg bw (mean of values for five rats)

Sample	Males	Females
Urine	78.6	81.9
Faeces	11.5	6.8
Contents of the gastrointestinal tract post mortem	<0.1	<0.1
Cage wash	2.1	1.6
Tissues (including carcass)	1.8	1.9
Total	94.1	92.3

From Brown (1997b)

Table 3. Tissue concentrations of radioactivity (µg pirimicarb equivalents/g) 4 days after administration of [^{14}C]pyrimidinyl-pirimicarb as a single oral dose at 1 mg/kg (mean of values for five rats)

Tissue	Males	Females
Brain	0.002	0.003
Gastrointestinal tract	0.006	0.007
Gonads	0.007	0.015
Heart	0.014	0.014
Kidneys	0.018	0.017
Liver	0.057	0.058
Lungs	0.018	0.019
Spleen	0.009	0.011
Abdominal fat	0.004	0.003
Bone	0.016	0.010
Muscle	0.008	0.009
Blood	0.045	0.046
Plasma	0.031	0.034
Residual carcass	0.013	0.016

From Brown (1997b)
The limit of detection was 0.0004 µg pirimicarb equivalents/g of tissue for all tissues analysed

At the end of the experiment, <2% of the administered dose remained in the tissues in both sexes, the highest concentration of radioactivity being in the liver (pirimicarb equivalents, <0.06 µg/g). All other tissue concentrations were lower than that present in plasma (pirimicarb equivalents, 0.03 µg/g). Less than 0.05% of the administered dose remained in the gastrointestinal tract contents in both sexes. There was no sex difference in tissue distribution of radioactivity (Brown, 1997b).

In an experiment similar to that described above (Brown, 1997b), the tissue distribution and excretion of radioactivity was studied in rats after administration [^{14}C]pyrimidinyl-pirimicarb as a single oral dose at 50 mg/kg bw (radiochemical purity, >98%). The results are presented in Tables 4 and 5.

Over 4 days, males eliminated a mean of 95% of the dose, of which >83% was in the urine and 10% in the faeces. Over the same period, females eliminated >93% of the dose, of which 74% was in urine and >16% was in faeces. The rate of excretion was rapid with >85% of the dose eliminated by males (79% in urine) and >76% by females (69% in urine) within 24 h of dosing. Excretion was essentially complete within 4 days, with <0.07% of the dose remaining in the gastrointestinal tract contents of both sexes. No pronounced sex difference was apparent in the rate of excretion.

Table 4. Recovery (% of administered dose) of radioactivity 4 days after administration of [^{14}C]pyrimidinyl-pirimicarb as a single oral dose at 50 mg/kg bw (mean of values for five rats)

Sample	Males	Females
Urine	83.6	74.1
Faeces	10.1	16.6
Terminal gastrointestinal tract contents	<0.1	<0.1
Cage wash	1.3	2.0
Tissues (including carcass)	2.1	1.7
Total	97.1	94.5

From Brown (1997c)

Table 5. Mean tissue concentrations of radioactivity (µg equivalents pirimicarb/g) 4 days after administration of [^{14}C]pyrimidinyl-pirimicarb as a single oral dose at 50 mg/kg (mean of values for five rats)

Tissue	Male	Female
Brain	0.163	0.124
Gastrointestinal tract	0.352	0.235
Gonads	0.549	0.514
Heart	1.026	0.557
Kidneys	1.272	0.740
Liver	1.914	1.638
Lungs	1.457	0.776
Spleen	0.602	0.423
Abdominal fat	0.216	0.164
Bone	0.508	0.290
Muscle	0.644	0.413
Blood	3.185	1.805
Plasma	2.354	1.130
Residual carcass	0.814	0.717

From Brown (1997c)
Limit of detection was 0.02 µg equivalent pirimicarb/g of tissue for all tissues analysed

When the rats were killed after 4 days, approximately ≤2% of the administered dose was found in the tissues for both sexes, the highest concentration of radioactivity being found in the liver (1.9 µg and 1.6 µg pirimicarb equivalents/g for males and females respectively). Concentrations in plasma were 2.4 µg equivalents/g in males and 1.1 µg equivalents/g in females; concentrations in all other tissues were lower. No marked sex difference was apparent in the tissue distribution of radioactivity (Brown, 1997c).

To determine whether repeated dosing affects the metabolic fate of pirimicarb, the tissue distribution and excretion of radioactivity was studied in groups of five male and five female AP_fSD rats given [^{14}C]pyrimidinyl-pirimicarb (radiochemical purity, >98%) as a single oral dose at 1 mg/kg bw. These rats had previously been given unlabelled pirimicarb (purity, 99.9%; reference No. Y00032/048/001) as 14 consecutive daily oral doses at 1 mg/kg bw. In all other respects, this experiment was similar to that described in Brown, 1997b. The results in male and female rats were essentially the same (Tables 6 and 7).

Table 6. Recovery (% of administered dose) of radioactivity 4 days after administration of [^{14}C]pyrimidinyl-pirimicarb as a single oral dose at 1 mg/kg bw to rats previously given unlabelled pirimicarb as 14 consecutive daily oral doses at 1 mg/kg bw (mean of values for five rats)

Sample	Males	Females
Urine	79.4	77.9
Faeces	14.8	15.0
Terminal contents of gastrointestinal tract	<0.1	<0.1
Cage wash	2.2	3.7
Tissues (including carcass)	2.1	2.0
Total	98.4	98.7

From Brown (1997d)

Table 7. Mean tissue concentrations of radioactivity (µg pirimicarb equivalents/g) 4 days after administration of [^{14}C]pyrimidinyl-pirimicarb as a single oral dose at 1 mg/kg bw to rats previously given unlabelled pirimicarb as 14 consecutive daily oral doses at 1 mg/kg bw (mean of values for five rats)

Tissue	Male	Female
Brain	0.002	0.002
Gastrointestinal tract	0.008	0.008
Gonads	0.008	0.013
Heart	0.016	0.013
Kidneys	0.023	0.020
Liver	0.062	0.058
Lungs	0.023	0.018
Spleen	0.011	0.011
Abdominal fat	0.003	0.003
Bone	0.008	0.007
Muscle	0.011	0.010
Blood	0.055	0.047
Plasma	0.040	0.033
Residual carcass	0.017	0.017

From Brown (1997d)
The limit of detection was 0.0004 µg pirimicarb equivalent/g of tissue for all tissues analysed

Over 4 days, both males and females eliminated a mean of 96% of the administered dose, with approximately 79% appearing in the urine and 15% in the faeces. The rate of excretion was rapid, with >80% of the administered dose being eliminated (71–74% in the urine) in both sexes within 24 h after dosing. Excretion was effectively complete after 4 days, with <0.1% of the administered dose being present in the gastrointestinal tract contents in both sexes.

When the rats were killed after 4 days, approximately ≤2% of the radiolabel was found in the tissues in both sexes, with the highest concentration of radiolabel being found in the liver (0.06 µg pirimicarb equivalents/g). Concentrations in plasma were 0.04 µg equivalents/g in males and 0.03 µg equivalents/g in females; concentrations in all other tissues were lower.

A comparison of the routes and rates of excretion and the tissue distribution of radioactivity after repeated dosing in this experiment with the corresponding results from rats given ^{14}C-labelled pirimicarb as a single dose at 1 mg/kg bw (Brown, 1997b) shows no marked differences, although a greater proportion of the radiolabelled dose was eliminated in the faeces after repeated dosing. A higher recovery of the administered dose was also seen in this study. Hence, the repeated administration of pirimicarb to rats at this dose appars to have no pronounced effect on excretion or tissue distribution profiles (Brown, 1997d).

1.2 Metabolism

The biotransformation of pirimicarb was investigated in groups of male and female AP$_f$SD rats given [^{14}C]pyrimidinyl-pirimicarb (radiochemical purity, >98%) as a single oral dose at either 1 or 50 mg/kg bw, or as a single oral dose at 1 mg/kg bw after repeated oral doses of unlabelled pirimicarb at 1 mg/kg bw (purity, 99.9%; reference No. Y00032/048/001). An additional group of bile-duct cannulated male and female rats were given ^{14}C-labelled pirimicarb as a single oral dose at 50 mg/kg bw. The structural identification of metabolites isolated from the urine, bile and faeces was made using mass spectroscopy, proton nuclear magnetic resonance spectroscopy and co-chromatography with reference standards. Metabolites were quantified by high performance liquid chromatography.

Male and female bile-duct cannulated rats excreted a mean of 64% and 54%, respectively, of the orally administered radioactivity in the urine, and 13% and 16% in the bile within 48 h (Table 8). The mean percentage recoveries in the faeces were <1% and 4% of the administered dose in males and females, respectively. These values are taken to represent the unabsorbed dose, which thereby indicate that absorption after oral administration was extensive.

Table 8. Mean recovery (%) of administered radioactivity over 48 h in pairs of bile-duct cannulated rats given ^{14}C-labelled pirimicarb as a single oral dose at 50 mg/kg bw

Sample	Males	Females
Urine	64.0	53.8
Bile	13.2	16.5
Faeces	0.8	3.6
Total	77.9	73.9

From Gledhill (1998)

In rats, pirimicarb was extensively metabolized, giving rise to 24 metabolites, of which 17 were identified. A proposed pathway for the biotransformation of pirimicarb in rats is presented in Figure 1, with the relative proportions of metabolites shown in Tables 9 to 11. The structures and names of the rat metabolites characterized are shown in Figure 2. The major proposed route of biotransformation was via hydrolysis of the carbamate function (leading mainly to the formation of carbon dioxide), to give the 4-hydroxypyrimidine (R31805, 062/06). This was either excreted directly, or as the glucuronide conjugate, or was *N*-demethylated to produce the most abundant metabolite, the *N*-methyl hydroxypyrimidine (R34865, 062/07), which was either *O*-conjugated with glucuronide or further *N*-demethylated to the amine (R31680, 062/14). All of these metabolites were excreted predominantly in the urine, accounting for a cumulative total of 46–69% of the administered dose, irrespective of dose or sex.

The excretion of a lower proportion of the administered dose as the glucuronide conjugates of R31805 (<3% in both sexes, as CTL III and CTL XVI) in bile-duct cannulated rats than in non-cannulated rats (10% and 14% in males and females, respectively) is attributed to the reabsorption of biliary hydroxypyrimidines and their subsequent glucuronidation and elimination in the urine. After hydrolysis of the carbamate moiety, other minor hydroxylated metabolites included metabolite CTL XVII and its *N*-demethylated product (6-hydroxymethyl R34865).

Table 9. Quantification[a] of metabolites in bile-duct cannulated rats given [^{14}C]pyrimidinyl-pirimicarb orally as a single nominal dose at 50 mg/kg bw

Metabolite No.	Males				Females			
	Urine	Bile	Faeces	Total	Urine	Bile	Faeces	Total
R34865	28	2	T	30	21	0	T	21
R31680	5	4	0	9	7	0	0	7
O-glucuronide conjugate of R31805 (CTL III)	—	—	—	—	1	1	T	2
R31805	10	0	T	10	15	1	T	16
6-hydroxymethyl R34865	7	0	0	7	—	—	—	—
CTL VI	4	0	0	4	—	—	—	—
6-hydroxymethyl R34865 + CTL VI	—	—	—	—	5	0	0	5
CTL VII	2	0	0	2	1	0	0	1
O-glucuronide conjugate of R34865	—	—	—	—	0	1	0	1
CTL IX	—	—	—	—	0	7	0	7
O-glucuronide conjugate of hydroxylated pirimicarb (062/01) (CTL X)	0	T	0	T	—	—	—	—
O-glucuronide conjugate of hydroxylated pirimicarb (062/01) (CTL XI)	0	2	0	2	0	2	0	2
R35140	0	T	0	T	—	—	—	—
O-glucuronide conjugate of carbamate R34836	0	T	0	T	—	—	—	—
CTL XIV	0	1	0	1	—	—	—	—
CTL XV	0	2	0	2	—	—	—	—
O-glucuronide conjugate of R31805	1	0	0	1	2	0	0	2
Hydroxylated R31805 (CTL XVII)	—	—	—	—	0	T	0	T
Unknown 1	T	T	T, NR	T	0	0	T, NR	T
Unknown 2	0	1	NR	1	0	T	NR	T
Unknown 3	3	1	NR	4	2	1	NR	3
Unknown 4	1	0	0	1	—	—	—	—
Unknown 10	1	T	0	1	T	0	0	T
Unknown 11	—	—	—	—	1	0	0	1
Unknown 12	—	—	—	—	1	T	0	1
Unknown 11 + 12	2	T	0	2	—	—	—	—
Totals	66	14	T	80	56	13	T	73

From Gledhill (1998)

T, trace level of metabolite; NR, could not be resolved as single metabolites

[a] Expressed as a percentage of the administered dose

Table 10. Quantification[a] of metabolites in male rats given ^{14}C-labelled pirimicarb as a single oral dose or as a single oral dose after 14 repeated doses of unlabelled pirimicarb

Metabolite No.	Single dose at 1 mg/kg bw			Repeated doses at 1 mg/kg bw			Single dose at 50 mg/kg bw		
	Urine	Faeces	Total	Urine	Faeces	Total	Urine	Faeces	Total
R34865	26	1	27	34	1	35	33	1	34
R31680	10	T	10	9	T	9	10	T	10
O-glucuronide conjugate of R31805 (CTL III)	2	T	2	5	T	5	3	0	3
R31805	6	T	6	8	T	8	12	1	13
6-hydroxymethyl R34865	2	T	2	6	T	6	6	0	6
CTL VI	11	T	11	4	T	4	5	0	5
CTL VII	3	0	3	5	0	5	1	0	1
O-glucuronide conjugate of R31805 (CTL XVI)	3	T	3	2	T	2	7	0	7
Unknown 1	4	NR	NR	4	2	6	1	1, NR	NR
Unknown 2	4	1, NR	9, NR	—	—	—	3	NR	5, NR
Unknown 3	1	0	1	—	—	—	—	—	—
Unknown 4	1	0	1	—	—	—	1	0	1
Unknown 5	3	0	3	—	—	—	—	—	—
Unknown 8	—	—	—	—	—	—	T	0	T
Unknown 10	0	T	T	0	T	T	2	T, NR	2
Unknown 11	—	—	—	—	—	—	0	NR	T
Unknown 12	—	—	—	—	—	—	—	—	—
Total	76	2	78	77	3	80	84	3	87

From Gledhill (1998)
T, trace level of metabolite; NR, could not be resolved as single metabolites
[a] Expressed as a percentage of the administered dose

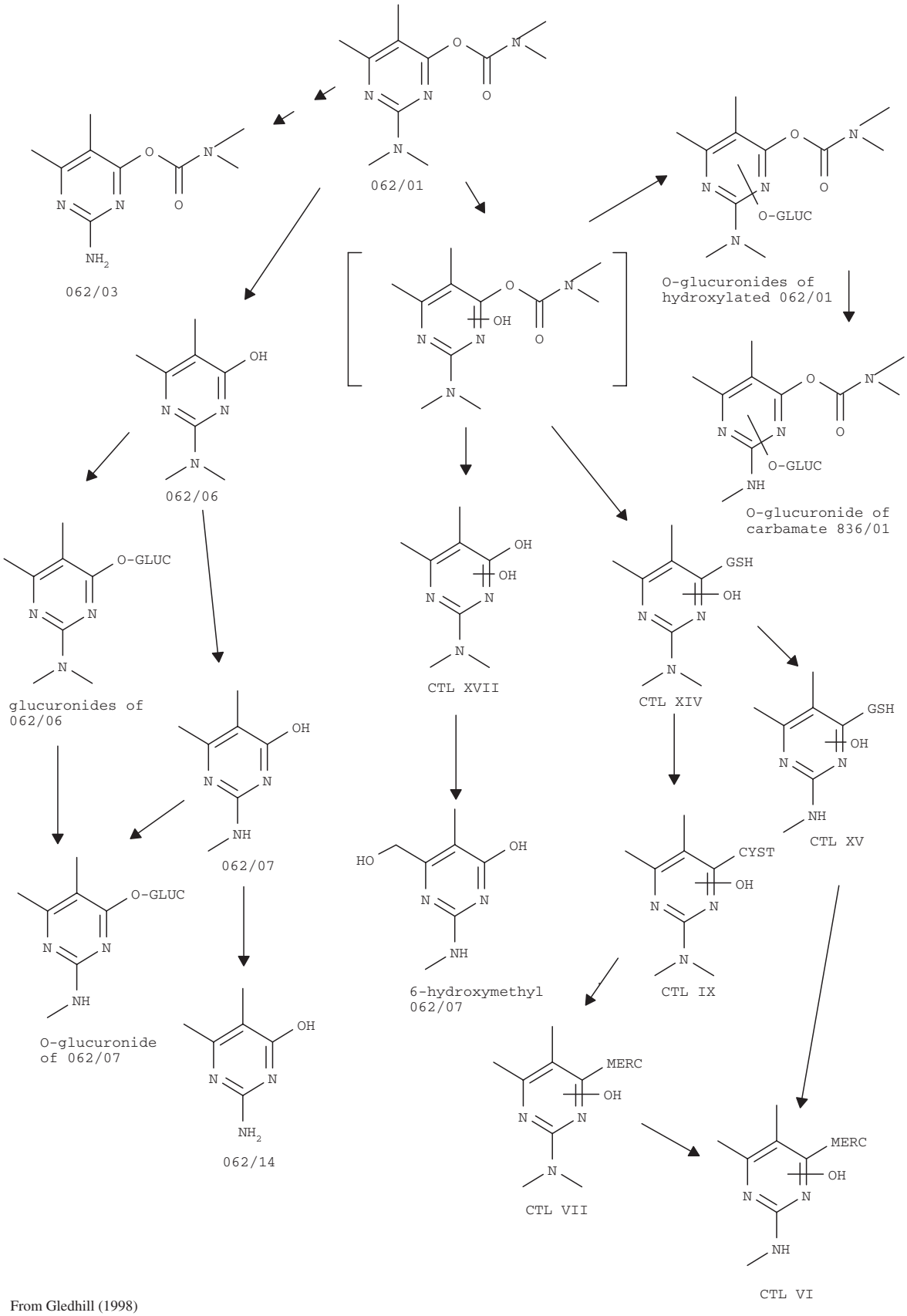
Table 11. Quantification[a] of metabolites in female rats given ^{14}C-labelled pirimicarb as a single oral dose only or as a single oral dose after 14 repeated doses of unlabelled pirimicarb

Metabolite No.	Single dose at 1 mg/kg bw			Repeated doses at 1 mg/kg bw			Single dose at 50 mg/kg bw		
	Urine	Faeces	Total	Urine	Faeces	Total	Urine	Faeces	Total
R34865	48	1	49	38	1	39	31	1	32
R31680	6	T	6	6	T	6	3	0	3
O-glucuronide conjugate of R31805 (CTL III)	8	T	8	9	1	10	4	T	4
R31805	6	T	6	4	0	4	15	1	16
6-hydroxymethyl R34865	4	T	4	3	T	3	4	0	4
CTL VI	3	T	3	4	1	5	2	0	2
CTL VII	5	0	5	3	0	3	3	0	3
O-glucuronide conjugate of R31805 (CTL XVI)	1	T	1	3	T	3	10	T	10
Hydroxylated R31805 (CTL XVII)	—	—	—	—	—	—	—	—	—
Unknown 1	1	NR	NR	2	2	4	1	NR	NR
Unknown 2	1	1, NR	3, NR	—	—	—	1	1, NR	3, NR
Unknown 3	—	—	—	—	—	—	—	—	—
Unknown 6	2	0	2	—	—	—	—	—	—
Unknown 7	1	0	1	—	—	—	—	—	—
Unknown 10–12	0	T	T	0	T	T	0	T	T
Totals	86	2	88	72	5	77	74	3	77

From Gledhill (1998)
T, trace level of metabolite; NR, could not be resolved as single metabolites
[a] Expressed as a percentage of the administered dose

The carbamate function was also subject to displacement by glutathione, which produced a number of metabolites. Hydroxylation of a ring methyl group followed by displacement of the carbamate moiety by glutathione produced metabolite CTL XIV, and its *N*-demethylated equivalent, metabolite CTL XV. In female rats, the intact glutathione

Figure 1. Proposed pathway for the biotransformation of pirimicarb (062/01) in rats



From Gledhill (1998)

Figure 2. Structure and nomenclature of characterized metabolites of pirimicarb in the rat

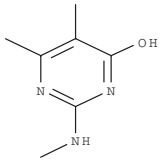
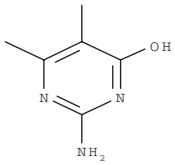
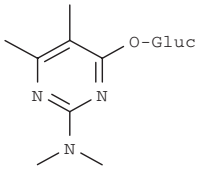
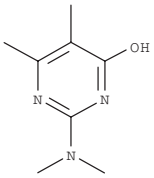
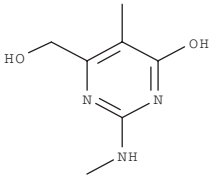
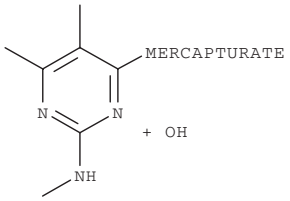
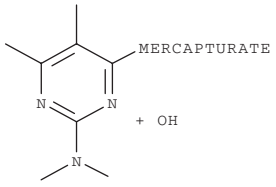
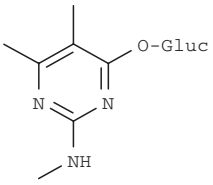
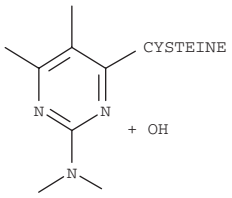
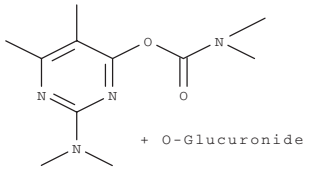
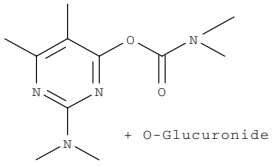
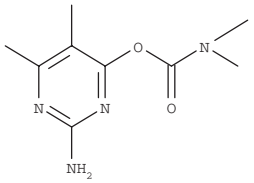
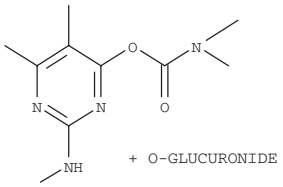
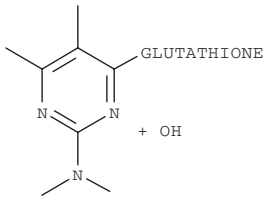
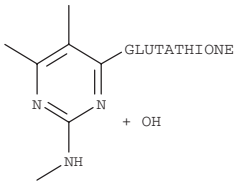
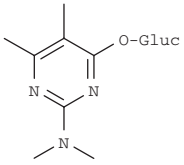
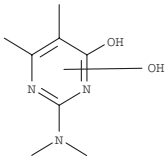
Metabolite No.	Structure and nomenclature	Metabolite No.	Structure and nomenclature
R34865 CTL I	 5,6-dimethyl-2-methylamino-4-hydroxypyrimidine	R31680 CTL II	 2-amino-5,6-dimethyl-4-hydroxypyrimdine
CTL III	 5,6-dimethyl-2-dimethylamino-4-hydroxypyrimidine glucuronide conjugate (*O*-glucuronide conjugate of R31805; isomer of CTL XVI)	R31805 CTL IV	 5,6-dimethyl-2-dimethylamino-4-hydroxypyrimidine
CTL V	 6-hydroxymethyl-2-methylamino-5-methylpyrimidin-4-ol (6-hydroxymethyl R34865)	CTL VI	
CTL VII		CTL VIII	 5,6-dimethyl-2-methylamino-4-hydroxypyrimidine glucuronide conjugate (*O*-glucuronide conjugate of R34865)

Figure 2. Continued

Metabolite No.	Structure and nomenclature	Metabolite No.	Structure and nomenclature
CTL IX	 hydroxylated 4-cysteinyl- 5,6-dimethyl-2-dimethyl aminopyrimidine	CTL X	 (*O*-glucuronide conjugate of hydroxylated pirimicarb (062/01); isomer of CTL XI)
CTL XI	 (*O*-glucuronide conjugate of hydroxylated pirimicarb (062/01); isomer of CTL X)	R35140 CTL XII	 2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate
CTL XIII	 (*O*-glucuronide conjugate of carbamate 836/01)	CTL XIV	
CTL XV		CTL XVI	 (*O*-glucuronide conjugate of R31805; isomer of CTL III)
CTL XVII	 (Hydroxylated R31805)		

From Gledhill (1998)

conjugate was not seen, but the corresponding cysteine conjugate (CTL IX) and mercapturate (CTL VII) were present. The *N*-demethylated mercapturate (CTL VI) was also present in the urine. There was a sex difference in the metabolic profile, with males showing a more extensive range of 4-hydroxypyrimidines than females.

While all urinary and faecal metabolites of [^{14}C]pyrimidinyl had lost the carbamate function, the analysis of samples of bile collected soon after dosing showed the presence of small amounts of R35140 (062/03) and the O-glucuronide conjugates of hydroxylated pirimicarb (062/01) and carbamate R34836 (836/01), both of which contained the intact carbamate moiety.

There were dose-dependent differences in the relative proportions of urinary metabolites, with more of the *N*-dimethyl 4-hydroxy metabolites R31805, 6-hydroxymethyl R34865 and O-glucuronide conjugate of R31805 and less of the *N*-demethylated metabolites R34865 and R31680 being found at 50 mg/kg bw than at 1 mg/kg bw. Rats at the highest dose excreted less of the methoxy-4-mercapturates, CTL VI and CTL VII, but the males excreted a higher proportion of the dose as 6-hydroxymethyl R34865.

There were fewer differences between the sexes in rats given repeated doses of pirimicarb than in rats given a single dose at 1 mg/kg bw, but the differences were not pronounced (Gledhill, 1998).

2. Toxicological studies

2.1 Acute toxicity

The acute oral toxicity of this pesticide was evaluated in groups of five male and five female fasted Alpk : AP$_f$SD rats given pirimicarb (purity, 97.6%; reference No. P16) at a dose of 100, 150 or 200 mg/kg bw by gavage in corn oil. The highest dose was selected on the basis of a preliminary study. The rats were observed for 14 days after dosing. At 100 mg/kg bw, there were no deaths and signs of slight toxicity were resolved by day 2. At 150 mg/kg bw, three males and three females died and slight signs of toxicity in the survivors were resolved by day 7. At 200 mg/kg bw, four males and all five females died; signs of mild toxicity in the surviving male were resolved by day 8. All gross pathological findings at autopsy were considered to be non-specific or incidental and not of toxicological importance. The acute oral median lethal dose (LD50) was calculated to be 152 mg/kg bw (95% CI, 118–197) in males and 142 mg/kg bw (95% CI, 121–166) in female rats (Lees & Connolly, 1995a).

In a study of acute percutaneous (dermal) toxicity, a group of five male and five female Alpk:AP$_f$SD rats was given pirimicarb (purity, 97.6%; reference No. P16) at a dose of 2000 mg/kg bw. Pirimicarb was mixed with approximately 0.5 ml of deionized water and applied at a dose of approximately 20–21 mg/cm^2 for males and 17–18 mg/cm^2 for females to the shaved intact skin, which was then covered by a gauze patch and an occlusive dressing for 24 h. The application site was then washed with water. Observations for mortality and clinical signs were made at 1 h and 4 h after application and then once daily for 14 days. Body weights were measured on days 1, 3, 5, 8 and 15. Autopsies were performed on all rats on day 15. No deaths occurred in this study and there were no significant signs of toxicity. There was never a significant loss in body weight in male rats and all males exceeded their initial body weight by day 5. Small body-weight losses were observed in female rats on day 3, but all females were gaining weight by day 8. Slight irritation of the skin

(including desquamation and small, scattered scabs) was noted in one male and two female rats. All dermal irritation had cleared by day 12. The dermal LD50 for pirimicarb was >2000 mg/kg bw in both male and female rats (Lees & Connolly, 1995b).

In a study of acute toxicity, groups of five male and five female Alpk:AP_fSD rats were given pirimicarb (purity, 97.4%; reference No. P18) by inhalation. Exposures were for 4 h to target atmospheric particulate concentrations of 400, 800 or 1200 µg/l. Analysed mean (± standard deviation) concentrations of pirimicarb were 414 ± 47, 747 ± 100 and 1065 ± 207 µg/l, respectively, and the corresponding mass median aerodynamic diameters (± geometric standard deviations) of the particles were 3.05 ± 2.04, 3.46 ± 1.89 and 3.02 ± 2.03 µm. No deaths occurred in the group with the lowest exposure. In the group with intermediate exposure, one female died, and in the group with the highest exposure, all rats either died or were killed in extremis. Clinical signs consistent with moderate toxicity and irritation to the respiratory tract were recorded in all treated rats either during or immediately after exposure. Surviving rats showed a rapid recovery from these effects. The median lethal concentration (LC50) of pirimicarb was 948 µg/l (95% CI, 746–1204) for male rats and 858 µg/l (95% CI, 703–1047) female rats (Parr-Dobrzanski, 1994).

The potential for pirimicarb (purity, 97.6%; reference No. P16) to cause acute ocular irritation was evaluated in six young male adult New Zealand White rabbits. Approximately 100 mg of pirimicarb was administered to one eye of each rabbit. The eyes were examined and assessed for irritation according to the Draize method. The rabbits were also assessed for possible initial pain response according to a six-point scale. There were no deaths in the study. Pirimicarb produced practically no initial pain (a few blinks only within 1–2 min) or slight initial pain (blinks and attempts to open the exposed eye, but the reflex closes it) in all six rabbits (class 1–2 on the six-point scale). Signs of irritation were limited to slight conjunctival redness in all rabbits and slight chemosis in one of them. These signs were no longer evident by day 2. The maximum mean total score (out of 110) was 2.3 at 2 h, reducing to 1.0 at 1 day and zero at 2 days. The final irritation assessment was that pirimicarb is practically non-irritant to the eyes of rabbits (Lees & Doyle, 1995c).

The acute dermal irritation potential of pirimicarb (purity, 97.6%; reference No. P16) was evaluated in six young male adult New Zealand White rabbits. Approximately 500 mg of pirimicarb was mixed with approximately 0.5 ml of deionized water and applied to the shaved intact skin (an area of approximately 2.5 cm × 2.5 cm), which was then covered by impermeable rubber sheeting for 4 h, after which the application site was exposed and washed with water. The Draize scale was used to assess the degree of erythema and oedema at the application sites approximately 30–60 min and 1, 2, 3 4, 7 and 12 days after removal of the dressings. Signs of skin irritation were limited to very slight erythema (score of 1 on a scale of 0 to 4) observed in two rabbits, one for up to day 2, the other for up to day 7. The latter rabbit showed desquamation on day 7, but not on day 12. There were no signs of irritation in any of the other rabbits. The Meeting concluded that pirimicarb is practically non-irritant to the skin of rabbits (Lees & Connolly, 1995c).

The potential of pirimicarb (purity, 97.3%; reference No. RS088/E) to produce delayed contact hypersensitivity in male Porcellus:Dunkin Hartley guinea-pigs was assessed by a method based on the maximization test of Magnusson & Kligman (1970). The doses of pirimicarb used were selected on the basis of a preliminary screen. Groups of 20 test and 10 control male albino guinea-pigs were used for the main study. Two main procedures were involved: (1) the induction of an immune response; and (2) a challenge to that response.

Guinea-pigs were induced by intradermal injection of the following: (1) Freund complete adjuvant plus corn oil at the ratio of 1 : 1; (2) a 3% w/v dilution of pirimicarb in corn oil; or (3) a 3% w/v dilution of pirimicarb in a 1 : 1 preparation of Freund complete adjuvant and corn oil. Three pairs of intradermal injections (each of 0.05–0.1 ml in volume) were made, one of each pair on either side of the dorsal midline. One week later, 0.2–0.3 ml of pirimicarb as a 75% w/v preparation in corn oil was applied topically, and retained under an occlusive dressing for 48 h. Guinea-pigs in the control group were treated in the same way, but without the inclusion of pirimicarb.

For the challenge, 2 weeks after completion of the induction phase, a 75% w/v preparation of pirimicarb in corn oil (volume, 0.05–0.1 ml) was applied to the shorn left flank of the test and control animals and held under an occlusive dressing for 24 h. A 30% w/v preparation of pirimicarb in corn oil (volume, 0.05–0.1 ml) was applied to the shorn right flank of test and control animals and held under an occlusive dressing for 24 h. Skin sites were examined approximately 1 and 2 days after removal of the dressings in both induction and challenge phases, and the degree of erythema was quantified and recorded. The sensitizing potential of a 40% solution of aqueous formaldehyde was assessed in a study using a method similar to that described above and serving as a positive control.

No response was seen in any of the control animals. The guinea-pigs treated with formaldehyde showed a net percentage response calculated to be 94%. Twenty-four hours after the removal of the dressings, six of the 19 test animals (the bandage slipped from one test animal that was therefore excluded from the analysis), showed scattered mild or moderate diffuse redness in response to the challenge application with a 75% w/v dilution of pirimicarb in corn oil. The net percentage response was calculated to be 32%. After challenge with a 30% w/v preparation of pirimicarb in corn oil, scattered mild redness to intense redness and swelling was seen in nine of the 19 test animals. The net percentage response was 47%. The Meeting concluded that pirimicarb does have skin sensitizing potential under the conditions of the maximization test (Rattray & Leah, 1990).

2.2 Short-term studies of toxicity

There were no short-term studies of toxicity in mice and no 28-day studies in rats.

Rats

In an 8-week feeding study, groups of 20 Wistar-derived female rats of the Alderley Park strain were fed diets containing pirimicarb technical (purity, 97.7%; reference No. BX 189) at a concentration of 0, 100, 175, 250 or 750 ppm of diet, equal to 0, 12.2, 20.4, 29.2 or 84.8 mg/kg bw per day for 8 weeks. The objective of this study was to establish a definite no-effect level for the depression in growth observed in previous 90-day (Griffiths & Conning, 1968), 2-year (Clapp et al., 1972), three-generation (Fletcher & Sothern, 1971) and paired feeding (Richards et al., 1978) studies in rats. The clinical condition of the rats was monitored throughout the study, while body weights, food consumption (including food wastage) and water consumption were measured weekly. Analysis of the diets confirmed that the achieved concentration and homogeneity of pirimicarb were satisfactory. Stability of pirimicarb in diet was confirmed in an earlier study.

There was no effect on the clinical condition of the rats and variations in water consumption were considered not to be compound-related. The body-weight gain of rats at 750 ppm was significantly lower than that of controls by week 3 and remained so until week

Table 12. Mean body-weight gain (g) at selected time-points in female rats fed diets containing pirimicarb for 8 weeks

Week	Dietary concentration (ppm)				
	0 (control)	100	175	250	750
1	31.0	28.6	32.7	29.7	28.0
4	94.4	91.2	93.0	93.4	82.8**
8	147.0	141.1	146.8	144.2	132.8**

From Paul et al. (1995)
** Statistically significant difference from appropriate control group mean, $p < 0.01$ (Student *t*-test)

Table 13. Overall mean food consumption and overall mean food wastage in female rats fed diets containing pirimicarb for 8 weeks

	Dietary concentration (ppm)				
	0 (control)	100	175	250	750
Food consumption (g)	1200	1215	1163	1172	1135**
Food wastage (g)	371.6	385.4	418.7	352.5	525.9**

From Paul et al. (1995)
** Statistically significant difference from appropriate control group mean, $p < 0.01$ (Student *t*-test)

8 (Table 12). Overall weight gain was slightly but not statistically significantly reduced for animals at 100 or 250 ppm compared with that of controls.

In rats fed diets containing pirimicarb at 750 ppm, there was a statistically significant overall reduction in food consumption (Table 13). There were some occasional statistically significant, sporadic reductions in food consumption at 175 and 250 ppm, but overall the difference from controls was not significant. There was no effect on water consumption or food utilization at any dose, but food wastage was statistically significantly increased in animals at 750 ppm. The effect on body-weight gain could therefore be caused by either toxicity or reduced food palatability. No distinction between these possibilities could be made from this experiment. The no-observed-adverse-effect level (NOAEL) for rats in this study was 250 ppm, equal to 29 mg/kg bw per day. However, in comparison with the four earlier studies cited above, this seems to be very close to a lowest-observed-adverse-effect level (LOAEL), so the clear NOAEL is 175 ppm, equal to 20 mg/kg bw per day (Paul et al., 1995).

In an 8-week feeding study, groups of 12 Wistar-derived female rats of the Alderley Park strain were given access ad libitum to diets containing pirimicarb technical (purity, 97.7%; reference No. BX 189) at a concentration of 0, 250 or 750 ppm, equal to 0, 27.5 or 89.1 mg/kg bw per day for 8 weeks. These treated rats were paired with four groups of rats fed restricted diets, the amount given to these rats being determined by the amount eaten by rats given access to food ad libitum. Rats given diets containing pirimicarb at 250 and 750 ppm ad libitum, equal to 27.5 and 89.1 mg/kg bw per day, respectively, were paired with groups of rats given restricted access to either control diet or diets containing pirimicarb at 250 or 750 ppm, equal to 25.7 and 75.0 mg/kg bw per day, respectively.

After 8 weeks of treatment, all groups were fed control diet ad libitum for a recovery period of 8 weeks. Throughout the study, body weights and food and water consumption

Table 14. Description of a feeding study in female rats fed diets containing pirimicarb for 8 weeks followed by an 8-week recovery period

Study part	Feeding regime	Group	Dietary concentration (ppm)
1	Ad libitum	1	0 (control)
		2	250
		3	750
2	Restricted to the amount of food eaten by the animals in group 2 (250 ppm, ad libitum)	4	0 (control)
		5	250
3	Restricted to the amount of food eaten by the animals in group 3 (750 ppm ad libitum)	6	0 (control)
		7	750

From Richards et al. (1978)

Table 15. Mean body-weight gain (g) at selected time-points from week 0 during the treatment period and from week 8 during the recovery period in female rats fed diets containing pirimicarb for 8 weeks followed by an 8-week recovery period

Week	Dietary concentration (ppm)						
	Feeding ad libitum			Restricted feeding			
	0 (control)	250	750	0 (control)	250	0 (control)	750
1	37.1	35.4	31.2**	28.5	24.3**	27.2	23.0**
4	101.8	100.3	89.3**	85.0	79.3*	79.0	73.6*
8	147.0	142.0	128.5**	132.4	127.3	120.3	117.7
9	6.9	6.3	8.5	8.7	11.5	12.7	14.8
13	27.2	29.9	33.8*	32.3	36.9	36.5	42.3
16	30.8	35.4	38.8	35.8	43.1	41.3	48.5

From Richards et al. (1978)
* Statistically significant difference from appropriate control group mean, $p < 0.05$ (Student t-test)
** Statistically significant difference from appropriate control group mean, $p < 0.01$ (Student t-test)

were measured periodically. Analysis of the diets confirmed that the achieved concentration and stability of pirimicarb were satisfactory throughout the study.

There was no effect on the clinical condition of the rats, and variations in water consumption were considered not to be compound-related. The effects on body weight and food consumption are described below, according to the feeding regime.

Ad libitum diets: There was a slight depression in growth at 250 ppm that was more marked at 750 ppm (Table 15). This reduction in growth was completely reversible after a recovery period of 8 weeks in rats previously fed at 250 ppm ad libitum, but although those fed at 750 ppm grew better than did the controls during the recovery period, they gained less weight overall. Food consumption and food utilization showed a dose-related reduction during the treatment period (Table 16). During the recovery period, food consumption of rats at 250 ppm was only marginally higher than that of the controls, but at 750 ppm was consistently higher from week 11 onwards. Food utilization tended to be more efficient and, at 750 mg/kg, differences were statistically significant from the controls (Table 17).

Restricted diets: body-weight gain was reduced at both 250 ppm and 750 ppm compared with that in the control group (Table 15). This was essentially confined to the first week, but the resultant difference in growth was maintained during the treatment period. This depression in growth was completely reversible during the 8-week recovery period. Food consumption in control and treated animals was marginally reduced during treatment

and was similar during recovery (Table 16). Food utilization was slightly less efficient than that of controls during the treatment period and was apparently improved (not statistically significant) during recovery (Table 17).

The Meeting concluded that there was no evidence that the reduced body-weight gain was caused by unpalatability of diets containing pirimicarb or as a result of anorexia. There was evidence that the growth depression was a toxic response, as the effect was immediate in all treated groups and was accompanied by poorer food utilization. Whilst the effect was only slight at 250 ppm, it supports the trends seen in previous long-term studies. The depression was completely reversible in rats fed restricted diets and in thoses fed at 250 ppm ad libitum, and almost so in animals fed at 750 ppm ad libitum (Richards et al., 1978).

Groups of 25 male and 25 female Alderley Park SPF rats were either fed diets containing pirimicarb (reference No. and purity not reported) at a concentration of 0, 250 or 750 ppm, equal to 12.9 or 38.8 mg/kg bw per day in males and 15.3 or 47.1 mg/kg bw per day in females, or were given pirimicarb at a dose of 25 mg/kg bw per day orally by gavage for 12 weeks, followed by a recovery period lasting until week 16. Body weights and food consumption were measured throughout the study. Blood was taken for haematological assessment from five rats of each sex per group before exposure and from all remaining rats at the end of the study. Before exposure and periodically during the exposure period, plasma and erythrocyte cholinesterase activities were measured. At the end of the study, cholinesterase activity was measured in brain samples from designated animals, and selected organs were weighed from five rats of each sex per group. A range of tissues from all animals was examined microscopically.

Table 16. Overall mean food consumption (g) in female rats fed diets containing pirimicarb for 8 weeks followed by an 8-week recovery period

Weeks	Dietary concentration (ppm)						
	Ad libitum feeding			Restricted feeding			
	0 (control)	250	750	0 (control)	250	0 (control)	750
1–8	1056	1052	1003*	946	942	904	894
9–16	1097	1082	1051	1061	1072	1060	1061

From Richards et al. (1978)
* Statistically significant difference from appropriate control group mean, $p < 0.05$ (Student *t*-test)
** Statistically significant difference from appropriate control group mean, $p < 0.01$ (Student *t*-test)

Table 17. Mean food utilization (g food/g weight gained) in female rats fed diets containing pirimicarb for 8 weeks followed by an 8-week recovery period

Weeks	Dietary concentration (ppm)						
	Ad libitum feeding			Restricted feeding			
	0 (control)	250	750	0 (control)	250	0 (control)	750
1–8	7.20	7.45	7.84**	7.16	7.45*	7.58	7.64
9–16	37.3	33.7	29.4*	35.9	27.5	27.1	23.4

From Richards et al. (1978)
* Statistically significant difference from appropriate control group mean, $p < 0.05$ (Student *t*-test)
** Statistically significant difference from appropriate control group mean, $p < 0.01$ (Student *t*-test)

Analysis of the diets showed that achieved concentrations were satisfactory throughout the study. There were no adverse effects of pirimicarb on survival or evidence of compound-related clinical changes. There was no clear evidence of an effect of pirimicarb on body weight, some statistically significant differences from the controls being inconsistent and not part of a dose–response relationship. Food utilization was statistically significantly reduced compared with that of the controls in females given diets containing pirimicarb at 750 ppm during weeks 1–4.

Statistically significant differences from control values were seen in several haematological parameters in rats treated with pirimicarb. These were, however, inconsistent and there was no evidence of a dose–response relationship in any of the changes seen.

Plasma cholinesterase activity was reduced in males given pirimicarb at 25 mg/kg bw by gavage during most weeks of the dosing period, but not during the recovery period (Table 18). The differences seen in males at weeks 15 and 16 were considered to be spurious as they occurred in the recovery period after a value similar to that of controls before termination. In females, plasma cholinesterase activity was reduced during all of the dosing period. Females at the lower doses were affected, but to a lesser degree. Reductions in plasma cholinesterase activity provide evidence for the absorption of pirimicarb.

There was no effect on brain cholinesterase activity. There were, however, decreases in erythrocyte cholinesterase activity at 25 mg/kg bw in males at weeks 2 and 4 and in females at weeks 1 and 4, and at 750 mg/kg in females in weeks 8 and 10 (Table 19). Although statistically significant differences from the control values were seen at some timepoints, the decreases were too small to be of toxicological significance.

There were no compound-related macro- or microscopic findings. There were no effects on organ weights (Griffiths & Conning, 1995).

It is concluded that no compound-related changes were seen in rats fed diets containing pirimicarb at 250 or 750 ppm. In contrast, plasma cholinesterase activity in rats

Table 18. Plasma cholinesterase activity (μmoles of acetic acid/ml per min) in rats receiving either diets containing pirimicarb or pirimicarb by oral gavage for 12 weeks followed by a 4-week recovery period[a]

Week	Dose or dietary concentration							
	Males				Females			
	0 (control)	250 ppm	750 ppm	25 mg/kg bw per day	0 (control)	250 ppm	750 ppm	25 mg/kg bw per day
Before exposure	0.57	0.59	0.64	0.56	2.32	3.03	2.86	2.43
1	0.54	0.52	0.53	0.55	3.28	2.49*	2.55*	2.38**
2	0.52	0.52	0.49	0.33**	2.48	2.32	1.99	1.63**
4	0.64	0.60	0.56	0.45**	2.90	2.40*	2.26**	1.26**
6	0.61	0.60	0.52	0.40**	3.16	2.77	2.83	2.21**
8	0.68	0.59	0.55*	0.47**	2.84	2.43	2.68	1.56**
10	0.61	0.61	0.48	0.63	3.85	3.06*	3.10*	2.14**
12	0.68	0.66	0.62	0.44**	3.56	3.13	3.08	2.29**
15	0.75	0.71	0.63*	0.71	4.13	4.15	3.68	3.85
16	0.79	0.69	0.56**	0.74	4.04	3.99	3.97	3.55

From Griffiths & Conning (1995)

[a] For animals tested during the recovery period (weeks 12 to 16), the adjusted mean values are shown for week 1 onwards)

* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

Table 19. Erythrocyte cholinesterase activity (μmoles of acetic acid/ml per min) in rats receiving either diets containing pirimicarb or pirimicarb by oral gavage for 12 weeks followed by a 4-week recovery period[a]

Week	Dose or dietary concentration							
	Males				Females			
	0 (control)	250 ppm	750 ppm	25 mg/kg bw per day	0 (control)	250 ppm	750 ppm	25 mg/kg bw per day
1	1.08	1.06	1.17	0.98	1.16	1.17	1.13	0.90**
2	1.30	1.42	1.46	1.16	1.24	1.36	1.37	1.43
4	0.92	0.99	1.01	0.79*	1.41	1.24	1.26	1.08*
8	1.11	1.02	1.08	0.96	1.34	1.22	1.16*	1.20
10	1.10	1.19	1.13	1.05	1.14	1.13	0.98*	1.09

From Griffiths & Conning (1995)

[a] For animals tested during the recovery period (weeks 12 to 16), the adjusted mean values are shown)

* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

receiving pirimicarb at a dose of 25 mg/kg bw per day by gavage was reduced if the examination was made within 4 h of the dose being given. Since feeding with diets containing pirimicab at 750 ppm resulted in a daily intake that was greater than that provided by administration of pirimicarb at a dose of 25 mg/kg bw per day by gavage, it is evident that the rapid absorption of pirimicarb can result in a C_{max} after dosing by gavage that is unobtainable from a diet containing pirimicarb at 750 ppm. Reductions in erythrocyte cholinesterase activity were too small to be of toxicological significance.

Groups of five male and five female rats received daily applications of pirimicarb (purity, 97.6%; reference No. P16) at a dose of 0, 40, 200, or 1000 mg/kg bw per day to the shaved, intact skin on 15 days (5 days per week) for 21 days. The test site was covered with gauze dressing followed by layers of stretch gauze and self-adhesive bandages. The exposure period was approximately 6 h/day. After treatment, the bandages were removed, and excess test substance was washed from the skin with water and mild soap. Body weight, food consumption, and clinical signs were evaluated throughout the study. Blood samples were collected before the rats were killed and subjected to examination post mortem at which selected organs were weighed and kidney, liver, treated and untreated skin, from animals in the control group and at the highest dose, were examined histopathologically.

None of the animals died before the scheduled end of the study and there were no signs of skin irritation, clinical signs of systemic toxicity or compound-related effects on body weight or food consumption. Also, there were no compound-related effects on organ weights, gross post-mortem or histopathological findings.

There were no compound-related effects on any haematological parameter.

At 1000 mg/kg bw per day, plasma alkaline phosphatase activity was reduced by about 20% in males ($p < 0.05$, Student *t*-test) and plasma cholesterol was about 34% higher in females ($p < 0.01$, Student *t*-test). There was a small but statistically significant reduction in brain cholinesterase activity at 1000 mg/kg bw per day, with no accompanying clinical signs of toxicity (Table 20). No statistically significant effects were observed at lower doses and there was no reduction of erythrocyte cholinesterase activity. There was a statistically significant reduction in plasma cholinesterase activity in males at 1000 mg/kg bw per day

Table 20. Cholinesterase activity (IU/g) in rats receiving pirimicarb by dermal application

Tissue	Dose (mg/kg bw per day)							
	Males				Females			
	0 (control)	40	200	1000	0 (control)	40	200	1000
Brain	8.35	7.73	7.47	6.81**	10.54	10.33	9.35	8.17**
Erythrocyte	2216	2314	2298	2200	2390	2270	2258	2168
Plasma	557	483	459*	448**	1228	1056	941**	741**

Lees & Leah (1995)
* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)
** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

and in females at 200 and 1000 mg/kg bw per day, but this was considered not to be of toxicological importance (Lees & Leah, 1995).

The NOAEL for dermal systemic toxicity was 200 mg/kg bw per day on the basis of reduced brain cholinesterase activity reduction at 1000 mg/kg bw per day.

Dogs

The report upon which this description is based contains information from two studies [referred to as part 1 (IHR241) and part 2 (IHR248)] that were combined because they were inter-related and had overlapping ranges of doses. Additional statistical analyses were carried out on both studies and included in this combined report as an addendum.

In part 1, groups of four male and four female beagle dogs were given diets delivering pirimicarb (purity, 94%; reference No. not reported) at a dose of 0, 4, 10 or 25 mg/kg bw per day for 90 days. Two males and two females from each group were then killed (one male only in the group at 25 mg/kg bw per day, as one male in this group became ill and died after only 10 weeks of treatment) and the remainder continued untreated for a further 28 days. Records were kept of their clinical condition, body weight, plasma, erythrocyte and brain cholinesterase activity, blood and urine chemistry, haematology (peripheral blood and bone marrow), terminal organ weights and histopathology.

In part 2, groups of four male and four female beagle dogs receivedpirimicarb at a dose of 0, 0.4, or 1.8 mg/kg bw per day for 90 days, while a fourth group received pirimicarb at 4.0 mg/kg bw per day for 180 days, the purpose being to determine a no-observed-effect level (NOEL) for the haematological changes seen in part 1. The observations and measurements made were similar to those made in part 1 but omitted the measurement of erythrocyte cholinesterase activity and the routine blood and urine chemistry.

In part 1, one male at 25 mg/kg bw per day became ill and was killed after 10 weeks of treatment, having lost 1.5 kg weight in 10 days and developed lethargy, urinary incontinence and severe anaemia. At post mortem, this dog was found to have abdominal ascites, a heavy nematode infestation of the ileum and congestion of the thymus, spleen and liver. It also had pronounced haematological abnormalities that are described below. The remaining dogs showed no clinical abnormality during the course of the experiment. There was a small compound-related reduction in body weight in males receiving pirimicarb at 25 mg/kg bw per day, with statistically significant differences from the values for controls occurring in most weeks between 5 and 14, the maximum difference being 6% ($p < 0.01$,

Student *t*-test) in week 14 (while in week 1 there had been a non-significant excess weight of almost 2%). There was no statistically significant effect on body weight among males at 10 or 4 mg/kg bw per day and no effects in females of either groups treated with pirimicarb.

During the course of the study, one male and one female at 25 mg/kg bw per day and one female at 10 mg/kg bw per day developed macrocytic anaemia. The male was killed (see above) and examination of the bone marrow post mortem showed marked erythropoietic hyperplasia with delayed maturation of the erythrocyte series and the presence of numerous megaloblasts. At this time (week 10), anaemia was not suspected in any of the other dogs. Statistical analysis of peripheral blood showed that there was an increase in mean erythrocyte diameter in both sexes at 25 mg/kg bw per day at 90 days (achieving statistical significance in females only) (Table 21). Circulating erythroblasts were markedly increased in individual dogs at 10 or 25 mg/kg bw per day, but the mean values did not achieve statistical significance. Examination of bone marrow in the recovery phase showed increases of myeloblasts and lymphocytes in males that had been receiving pirimicarb at a dose of 10 mg/kg bw per day and in the normoblast population in the males that had been receiving pirimicarb at 25 mg/kg bw per day (Table 22). In both males and females, there were increases (at all doses) in megaloblast counts both before and after recovery, but the data were erratic and showed no indication of a dose-related response. An interpretation of this observation is not evident.

There was a reduction in plasma cholinesterase activity at 25 mg/kg bw per day compared with that for controls, the maximum reductions being 30% at week 2 in male and 28% at week 1 in females (Table 23). Activity was sporadically reduced at 10 mg/kg bw per day, but there were no effects at 4 mg/kg bw per day. There was evidence of a reduction in

Table 21. Selected haematology parameters[a] at day 90 in dogs fed diets containing pirimicarb ("part 1")

Parameter	Dose (mg/kg bw per day)							
	Males				Females			
	0 (control)	4	10	25	0 (control)	4	10	25
Erythrocyte diameter (μm)	6.00	6.09	6.03	6.22	6.03	6.06	6.14	6.34*
Circulating erythroblasts/500 leukocytes	0.5	2.5	3.0	16.5	0.0	1.5	42.0	27.3

From Hodge (1995a)
* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)
[a] Adjusted mean values

Table 22. Bone-marrow differential cell counts[a] in dogs fed diets containing pirimicarb ("part 1")

Parameter	Dose (mg/kg bw per day)							
	Males				Females			
	0 (control)	4	10	25	0 (control)	4	10	25
Myeloblasts	0.7	0.8	2.3**	1.2	1.0	1.1	1.5	0.8
Lymphocytes	1.5	2.0	6.8**	1.7	4.2	3.6	1.0*	2.1
Early normoblasts	4.6	6.3	5.8	10.5**	4.1	6.1	4.8	7.2
Megaloblasts	3.8	13.7	8.2	16.2	1.8	24.4*	10.3	15.1

From Hodge (1995a)
[a] Recovery phase; adjusted mean values
* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)
** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

Table 23. Blood plasma cholinesterase activity[a] (µmol/ml per min) in dogs fed diets containing pirimicarb ("part 1")

Week	Dose (mg/kg bw per day)							
	Males				Females			
	0 (control)	4	10	25	0 (control)	4	10	25
1	2.25	2.22	1.83**	1.61**	2.17	2.14	1.76**	1.57**
2	2.15	2.16	1.83*	1.50**	2.18	2.02	1.71*	1.82*
4	2.46	2.46	2.25	2.10	2.54	2.30	1.97*	2.11*
6	2.48	2.67	2.11	1.98*	2.59	2.32	1.96	1.98*
8	2.61	2.52	2.06	2.52	2.11	2.19	2.09	2.54
10	3.01	2.59	2.45	1.92*	2.82	2.75	1.86	1.87
12	2.78	2.74	2.29	1.82**	2.60	2.90	2.55	2.12
Recovery period								
1	2.92	2.94	2.83	3.69**	2.83	2.67	2.83	3.50
4	2.68	2.59	2.57	2.84	2.80	2.71	3.07	2.79

From Hodge (1995a)
[a] Adjusted mean values shown for week 2 onwards.
* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)
** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

Table 24. Erythrocyte cholinesterase activity[a] (µmol/ml/min) in dogs fed diets containing pirimicarb ("part 1")

Week	Dose (mg/kg bw per day)							
	Males				Females			
	0 (control)	4	10	25	0 (control)	4	10	25
6	1.73	1.70	1.58	1.44	2.06	2.03	1.86	1.80
8	1.73	1.69	1.65	1.35	1.62	1.98	2.09	1.69
10	1.91	1.72	1.69*	1.37**	2.10	1.92	1.71*	1.58*
12	1.95	1.74	1.63	1.42**	2.09	1.99	1.89	1.65**

From Hodge (1995a)
[a] Adjusted mean values
* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)
** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

erythrocyte cholinesterase activity in the groups receiving pirimicarb at a dose of 10 and 25 mg/kg bw per day between weeks 6 and 12 in males and at weeks 10 and 12 in females. There was no effect at 4 mg/kg bw per day (Table 24).

There appeared to have been a compensatory increase in both plasma and erythrocyte cholinesterase activity after cessation of dosing. There was no evidence for a reduction in brain cholinesterase activity.

No compound-related effects on the blood and urine chemistry were observed.

No toxicologically significant reductions or increases in organ weights were observed. An apparent reduction in adrenal weight adjusted for body weight in females at 25 mg/kg bw per day was attributable to one unusually high value among the controls.

Extra-medullary haematopoiesis was observed in the spleens and lymph nodes of dogs with severe anaemia. Treated dogs tended to show a higher incidence of focal

inflammatory lesions in the liver and reactive changes in lymph nodes, but the differences were not marked.

In part 2, there was no evidence of any compound-related effect on clinical condition or body weights. Also, there was no evidence for any adverse effect on the haematological parameters measured at 0.4 or 1.8 mg/kg bw per day. However, serum iron levels were significantly reduced in females at 4 mg/kg bw per day at 60 and 90 days (Table 25).

Changes in the bone marrow were observed in the group receiving a dose of 4.0 mg/kg bw per day at 90 and/or 180 days (Table 26). These included increases in myelocytes in males; decreases in neutrophils in males and females; and increases in megaloblasts in both sexes at all time-points. It is noted that there was a substantial difference in the values for megaloblasts in the control groups in part 1 and part 2.

There was no evidence of inhibition of either plasma or brain cholinesterase.

Table 25. Serum concentrations of iron[a] (mg/100 ml) in female dogs fed diets containing pirimicarb ("part 2")

Period	Dose (mg/kg bw per day)			
	0 (control)	0.4	1.8	4.0
Before dosing	132.2	104.3	128.7	130.4
30 days	143.3	133.0	156.9	110.8
60 days	192.0	160.1	128.9	104.0*
90 days	198.3	150.5	139.8	114.0**

From Hodge (1995a)
[a] Adjusted mean values shown
** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)
* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

Table 26. Bone marrow differential cell counts[a] in female dogs fed diets containing pirimicarb ("part 2")

Parameter/period	Dose (mg/kg bw per day)							
	Males				Females			
	0 (control)	0.4	1.8	4.0	0 (control)	0.4	1.8	4.0
Myelocytes								
90 days	2.6	3.2	1.7	5.0**	3.8	3.5	4.3	4.7
180 days	—	—	—	4.9	—	—	—	3.2
Neutrophils								
90 days	18.7	16.5	16.6	12.1*	14.0	13.6	12.1	10.9
180 days	—	—	—	9.2	—	—	—	9.1
Megaloblasts								
30 days	0.6	2.0	1.4	3.7**	0.6	1.2	1.5	3.4**
60 days	0.9	1.8	1.7	4.2**	0.8	1.3	1.9	5.4**
90 days	0.8	1.1	3.0	6.6**	0.5	0.9	1.4	4.9**
180 days	—	—	—	7.3	—	—	—	6.4

From Hodge (1995a)
[a] Adjusted mean values shown except at 180 days.
* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)
** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

The only difference in organ weights compared with control values was a decrease in liver weight, adjusted for body weight, in males in all treatment groups. There was no dose–response relationship and the differences appear to result from two high control values.

The gross and microscopic findings showed no abnormalities attributable to treatment. There was evidence of splenic haematopoiesis, such as occurred in part 1, in only two dogs maintained for 180 days at 4 mg/kg bw per day. The NOAEL for pirimicarb was 1.8 mg/kg bw per day on the basis of haematological toxicity at 4.0 mg/kg bw per day (Hodge, 1995a).

Two groups of dogs were given diets containing pirimicarb (purity, 98.0%; reference No. Bx189 Dope D) as a suspension in Tween 80 for up to 16 weeks. One group of one male and one female, received pirimicarb at a dose of 2 mg/kg bw per day throughout this period. The second group of three males and three females, received pirimicarb at a dose of 25 mg/kg bw per day, increased to 50 mg/kg bw per day in two animals over weeks 5 and 6, and in all dogs of this group during weeks 8–12. A third group of one male and one female served as a control and received only Tween 80 in the diet. All groups were maintained on normal diet for 7 weeks after the dosing period. The condition and behaviour of all dogs was monitored periodically during the day. Body weights were recorded weekly and daily food intake was measured by weighing any uneaten food. Blood was taken for haematological investigations twice before the dosing period and then weekly for the remainder of the study. Bone marrow biopsies were performed on all dogs once before the dosing period and at 2-week intervals for the remainder of the study. Serological investigations were performed on all dogs at weekly intervals throughout the study. Erythrocyte and plasma cholinesterase activities were measured in all dogs on four (weekly) occasions before the dosing period, on day 1 of treatment (at 1, 2, 3, 4, 5 and 6 h) and subsequently at weekly intervals throughout the study. Clinical chemistry parameters were examined twice before the dosing period and then after 6 and 13 weeks. At the end of the study, 6 out of 10 dogs were killed and subjected to examination post mortem. Selected organs were weighed and a comprehensive range of tissues were taken and stored.

One dog was killed after 43 days of the study (28 days at 25 mg/kg bw per day, 10 days at 50 mg/kg bw per day and not dosed for 5 days) after clinical deterioration over the preceding 14 days. Autopsy revealed the probable cause of the deterioration as an intussusception of the ileum. (An intussusception is a form of intestinal obstruction caused by the prolapse of a part of the intestine into the adjoining intestinal lumen). It was not possible to establish any causal relationship between treatment and this condition. Salivation and a general loss of condition was associated with treatment in some dogs at 50 mg/kg bw per day. There were minimal changes in condition in one animal at 25 mg/kg bw per day and no changes were seen at 2 mg/kg bw per day. Body-weight gain was adversely affected in males at 25 mg/kg bw per day over the first 4 weeks of the study and in all dogs when the dose was increased to 50 mg/kg bw per day. Food intake was slightly reduced and feeding behaviour was affected at 50 mg/kg bw per day. It was not clear whether these effects were attributable to toxic effects of pirimicarb or to a decreased palatability of the diet.

Anaemia and reticulocytosis were associated with treatment at 50 mg/kg bw per day, as shown by reduced haemoglobin, erythrocyte volume fraction and erythrocyte count and an increase in the reticulocyte count of some dogs (Table 27). Responses to reduction of the dose to 25 mg/kg bw per day or to cessation of dosing varied, but generally there were returns towards, or to, control. Reticulocytes tended to increase in dogs M 693 and F 699 before they decreased once more.

Table 27. Haematology data (selected parameters) at week 12 in dogs fed diets containing pirimicarb

Dose/Dog No.	Parameter			
	Haemoglobin (g/dl)	Erythrocyte volume fraction (%)	Erythrocyte count (10^6/mm^3)	Reticulocyte (%)
Control				
M 691	13.9	45	6.89	0.6
F 696	13.9	47	6.27	0.6
2 mg/kg bw per day				
M 692	14.6	51	6.80	0.8
F 697	15.5	51	6.98	0.6
25/50 mg/kg bw per day				
M 693	D	D	D	D
M 694	3.8	15	2.16	0.4
M 695	13.1	47	5.50	2.6
F 698	13.3	43	5.76	3.8
F 699	9.7	31	4.04	0.8
F 700	12.1	37	5.02	3.2

From Fox (1998)
D, dead; F, female; M, male

At 50 mg/kg bw per day, changes in bone marrow were associated with an increase in the number of normoblasts from week 9 and a tendency towards suppression of bone marrow activity (hypoplasia). These effects were reversed when the dose was reduced to 25 mg/kg bw per day (after week 12) and all marrow samples were considered normal 1 week after cessation of treatment.

Inhibition of plasma cholinesterase activity compared with control values reached up to 85% at 25 mg/kg bw per day and >90% at 50 mg/kg bw per day. This effect was reversed within 7 days after cessation of treatment. Erythrocyte cholinesterase activity was not affected by treatment at any dose. There were no changes in other clinical pathology parameters.

Both absolute and relative spleen weights were at least fourfold greater in all treated females, while the only control female had a comparatively small spleen. Owing to the small number of dogs in the study and the lack of background data on these dogs, it was not possible to determine whether the changes were truly associated with treatment, although this would seem to be a reasonable assumption. It was considered that the changes in spleen weight were probably a reflection of the haematological changes described above. On the other hand, the splenic weight differences were also seen in the group receiving pirimicarb at 2 mg/kg bw per day, in which there were no signs of anaemia.

The results of this study demonstrate a rapid drop in haemoglobin concentration and a rise in reticulocyte numbers in two animals at 50 mg/kg bw per day. These changes, typical of haemolytic anaemia, are similar to those seen in previous studies in beagle dogs. The changes in the bone marrow appearances correlated well with the changes in peripheral blood haematology and plasma cholinesterase activity was markedly inhibited at 25 and 50 mg/kg bw per day indicating an expected biological response to treatment. The response of both dogs that received 2 mg/kg bw per day was in general similar to those seen in control dogs indicating that in this study, 2 mg/kg bw per day might be an NOAEL (Fox, 1978). Nevertheless, it could also be argued that, given the uncertainties regarding observations on the spleen, this study is unsuitable for reaching any such conclusion.

Groups of four male and four female beagle dogs were given gelatin capsules containing pirimicarb (purity, 97.5%; reference No. P22) orally at a dose of 0, 3.5, 10 or 35/25 mg/kg bw per day for a period of at least 1 year. The initial high dose of 35 mg/kg bw per day was given for 1 week, after which dosing was suspended for 2 weeks and the dose then reduced to 25 mg/kg bw per day from week 4 for the remainder of the study.

Clinical observations (including ophthalmoscopy) were made and body weights, food consumption and clinical pathology parameters, including haematology and plasma and erythrocyte cholinesterase activity, were measured throughout the study. At the end of the dosing period, the dogs were killed and subjected to a full post-mortem examination. Bone marrow smears were taken at this time. Selected organs were weighed, brain cholinesterase activity was measured and specified tissues were taken for histopathology.

Pirimicarb administered at the initial high dose of 35 mg/kg bw per day produced clear, clinical signs of toxicity (including tremors, salivation, thin appearance, sides pinched in, unsteady gait, subdued behaviour, irregular breathing and/or occasional coughing) and a significant reduction in appetite and subsequent body-weight loss during week 1. One female at 35/25 mg/kg bw per day was killed for humane reasons in week 36 after significant body-weight loss and moderately severe anaemia. Also at this dose of 35/25 mg/kg bw per day, there were clinical signs of toxicity, e.g. tremors, thin appearance, irregular breathing and coughing, a marginally increased incidence of fluid faeces and reductions in body-weight gain (46% in males, 23% in females over 52 weeks) and food consumption. The body-weight gain deficits were statistically significant over weeks 2–24 ($p < 0.01$) in males and at week 2 ($p < 0.01$) in females. The reductions in food consumption did not appear to correlate well with the reduced body-weight gain, in that they occurred with statistical significance erratically throughout the study in females, but were statistically significant in males only in week 1. Slightly reduced food consumption was also seen in several females receiving pirimicarb at a dose of 10 mg/kg bw per day. However, in the absence of any treatment-related effects on body weight at this dose, this was considered to be of no toxicological significance.

There were no treatment-related effects that were detected by ophthalmoscopy.

One bitch at 25 mg/kg bw per day developed anaemia that was first observed at week 13 and progressed until, at week 36, the dog was killed for humane reasons. Increased bone marrow cellularity and a decreased myeloid : erythroid ratio indicative of increased erythropoietic activity was also seen in this animal. No other significant haematological or bone-marrow effects were observed in dogs of any group.

Increased erythropoietic activity of the bone marrow was seen in the female dog that was killed in week 36. In those dogs that survived to the end of the study, there were no treatment-related changes in haematology or urine chemistry. At a dose of 35/25 mg/kg bw per day, there were slightly reduced plasma concentrations of albumin (in weeks 13, 26 and 52, $p < 0.05$, for males; in week 26 for females, $p < 0.01$) and total protein (in week 13, $p < 0.01$, and week 53, $p < 0.05$, for males; in week 26 for females, $p < 0.05$).

There were no changes in plasma, erythrocyte or brain cholinesterase activities that were attributable to treatment with pirimicarb in the female dosed at 35/25 mg/kg bw per day that was killed for humane reasons during week 36. There was no evidence from any of the surviving dogs of an adverse effect on plasma cholinesterase activity throughout the

study. Erythrocyte cholinesterase activity in females receiving pirimicarb at 35/25 mg/kg bw per day was statistically significantly lower (by approximately 21%) than concurrent control values in week 52, when adjusted for values obtained before dosing (Table 28). In addition, brain cholinesterase activity for females at 25 mg/kg bw per day, was statistically significantly lower (by approximately 22%) than concurrent control values in week 53. For males receiving pirimicarb at 25 mg/kg bw per day, and for males and females at 3.5 or 10 mg/kg bw per day, there were no changes in erythrocyte or brain cholinesterase activity that could be attributed to administration of pirimicarb.

There were no treatment-related changes in organ weights or macroscopic findings.

Histopathological changes consistent with an increase in erythrocyte breakdown (including increased haemosiderin pigmentation in the liver, extramedullary haematopoeisis in the spleen and bone marrow hyperplasia) were seen in the female dog that was killed in week 36. In the remaining dogs in the group treated with pirimicarb at 35/25 mg/kg bw per day and that survived to week 53, there was increased haemosiderin pigmentation of the liver in three out of four males and increased haemosiderin pigmentation of the spleen in one out of four males and one out of three females. Increased haemosiderin pigmentation of the spleen only was also apparent in one out of four females that received pirimicarb at a dose of 10 mg/kg bw per day. The NOAEL was 3.5 mg/kg bw per day on the basis of haemosiderin deposition in the liver and spleen at 35/25 mg/kg bw per day and in the spleen at 10 mg/kg bw per day (Horner, 1998).

Groups of four male and four female beagle dogs were fed pirimicarb (reference No. and purity not reported) at a measured dose of 0, 0.4, 1.8 or 4.0 mg/kg bw per day continuously for 2 years. The animals were observed daily and detailed clinical examinations were made at intervals of 3 months. Body weights were measured weekly for the first 12 weeks and then monthly throughout the remainder of the study. At 3-month intervals, blood samples were taken for haematology, cholinesterase activity and biochemistry; urine was collected for analysis; and electrocardiography readings were taken. Bone marrow samples were taken and examined at 4–6-month intervals. At the end of the exposure period, the dogs were killed and subjected to a full post-mortem examination. Selected organs were weighed and specified tissues were taken from all groups for subsequent histopathological examination. Histopathological examination of the brain and spinal cord was undertaken

Table 28. Cholinesterase activity (U/l) in dogs fed capsules containing pirimicarb for at least 1 year

Tissue	Dose (mg/kg bw per day)							
	Males				Females			
	0	3.5	10	35/25	0	3.5	10	35/25
Erythrocytes								
Before dosing	2823	3360	2500	2713	3010	2528	2843	2508
Week 52	3068	3850	2553	2818	3435	2728	3060	2633
Week 52, adjusted[a]	3095	3309	2921	2962	3253	2942	3015	2579**
Brain								
Week 52	4.82	5.00	4.84	4.48	5.68	5.52	5.11	4.43*

From Horner (1998)

[a] Adjusted (analysis of covariance) for values obtained before dosing

* Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

** Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

on animals in the control group and in the group receiving pirimicarb at a dose of 4.0 mg/kg bw per day only.

All dogs survived the exposure period and maintained a satisfactory standard of clinical health throughout. There were no adverse changes in the growth rates of the dogs.

No changes were found in plasma glucose, urea, sodium or potassium concentrations, alkaline phosphatase activity, or bromosulfthalein (BSP) retention times. No changes were detected in the urine analyses. Apart from a slight increase in the erythroid : myeloid ratio in two females at 4 mg/kg bw per day, no adverse changes were seen in the bone marrow and there were no abnormalities in the haematological parameters measured. No significant differences in serum iron or folate levels were detected between any of the groups.

No adverse effects of treatment were seen at post-mortem examination and no histopathological changes attributable to treatment were identified. There was no effect on organ weights. The NOAEL was 1.8 mg/kg bw per day on the basis of slight increases in erythroid:myeloid ratio, indicative of increased erythropoiesis, in two out of four females at 4.0 mg/kg bw per day (Hodge, 1995b).

In an earlier study, pirimicarb was found to produce anaemia when fed to pure-bred dogs (Conning et al., 1968). Of the 24 dogs given this compound, three became anaemic and 14 others had detectable changes in their bone-marrow cells. Subsequent studies involving another 48 dogs reproduced some of the bone-marrow effects, but none of the dogs became anaemic. The bone-marrow effects were not progressive and the incidence was much lower than in the first study (Conning et al., 1969; Garner et al., 1972).

Initial observations on the three anaemic dogs suggested that the anaemia was of the megaloblastic type, thus implying some interference with nucleic acid synthesis. A number of drugs, particularly the anticonvulsants, are known to produce megaloblastic anaemia in man by interfering with the absorption and metabolic activities of vitamin B12 and folic acid (Kidd & Mollin, 1957; Flexner & Hartman, 1960, MacIntosh & Hutchinson, 1960; Huennekens, 1966; Reynolds et al., 1965). Overt anaemia is uncommon in human patients receiving these drugs, but about one third show changes in their bone-marrow cells that are indicative of megaloblastic erythropoiesis (Chanarin, 1969). Administration of folic acid or folinic acid at large doses of invariably corrects the anaemia and abolishes the bone-marrow irregularities (Chanarin, 1969).

Megaloblastic erythropoiesis also develops in association with a number of haematopoietic disorders, especially haemolytic anaemia (Dacie, 1962). Conversely, intramedullary haemolysis is commonly encountered in classical megaloblastosis caused by to vitamin B12 or folate deficiency (Dacie, 1967a).

When the study described below (Garner et al., 1995) was begun, it was considered that the most likely explanation for the anaemia was an abnormality of erythropoiesis. Normal plasma levels of folate and vitamin B12 excluded the possibility that the absorption from the gut was impaired, but an effect at the cellular level still had to be considered. The absence of changes in leukocytes and platelets was contraindicative, and other studies with rats, guinea-pigs and ferrets had also failed to produce anaemia or bone-marrow changes (Griffiths & Conning, 1968; Clapp et al., 1972; Garner, 1972; Garner & Smith,

1972). In the study described below, the complete failure to correct the anaemia displayed by the two affected dogs with high doses of vitamin B12, B6, folic acid and folinic acid endorses the conclusion that the anaemia is not primarily megaloblastic.

The incidence of anaemia and the declining frequency of bone-marrow changes in the three reported studies in dogs raised the possibility of an idiosyncratic defect. Family studies showed that all dogs used in the first 90-day experiment were the offspring of five females, three of which were mated to the same male. The discovery of congenital factor VII deficiency (Garner et al., 1967) led to changes in the dog breeding policy and a substantial number of new breeding stock were introduced to eliminate this blood coagulation defect. Consequently, dogs used in studies after 1969 differently intrinsically from those in earlier studies. In the study described below, no evidence of a erythrocyte metabolic defect or abnormal haemoglobin structure was found in any of the four dogs used in the study or in a number of untreated stock dogs. There was, however, no doubt that the antibody detected in their sera was related to the administration of pirimicarb: it was not present before dosing; serial studies showed a dose-response relationship; and withdrawal of pirimicarb resulted in the disappearance of the antibody within 6 weeks.

Harris (1954) described a drug-induced haemolytic anaemia resulting from a erythrocyte-drug-antibody reaction in patients given the schistosomicide, Stibophen. Since then, a number of other drugs have been reported to cause haemolytic anaemia by this mechanism. All of the earlier literature relate to studies in human patients. Drug-induced autoimmune anaemia in man may be broadly classified into two types:

Type A: Haemolysis is acute and intravacular, resulting in haemoglobinaemia and haemoglobinuria. The required dose is low and there is usually some evidence of previous sensitizing exposure. Gamma-globulin cannot usually be detected on the erythrocyte surface, but components of complement can be demonstrated with suitable antisera.

Type B: Haemolysis is chronic and essentially extravascular. Haemoglobin degradation products may be present in plasma. Free haemoglobin cannot usually be detected either in plasma or urine. Large doses of the drug are required to produce an effect. Anaemia takes several weeks to develop. There is no evidence of sensitization, i.e. normal therapeutic doses of the drug can subsequently be given without adverse effect. Gamma-globulin can always be detected on the surface of circulating erythrocytes. Normal erythrocytes cannot be substituted for those of an affected person in vitro despite the presence of the drug. Antibodies are usually IgG immunoglobulins, but mixed IgM/IgG immunoglobulins are occasionally encountered.

Erythrocytes coated with IgG (auto-) antibodies are removed by the reticuloendothelial system. These antibodies may arise spontaneously or in association with diseases (systemic lupus erythematosus (SLE), lymphoma, chronic lymphocytic leukaemia) or after stimulation by a drug (e.g. α-methyldopa, l-dopa). The diagnostic laboratory hallmark is a Coombs' (antiglobulin) test, and the autoantibodies may be related to a portion of the Rhesus (Rh) locus (demonstrated for α-methyldopa; Bakemeier & Leddy, 1968). High-dose penicillin or cephalosporins may result in an antibody directed against an antibiotic–erythrocyte membrane complex; cessation of exposure to the drug results in the disappearance of accelerated destruction.

Pirimicarb-induced haemolytic anaemia could be included in the type B category, which also includes penicillin and α-methyldopa, the more common causes of this effect (Bunn & Rosse, 2001). Both require exposure at very high doses and for a protracted period to produce the effect, and both stimulate the production of IgG, but while the presence of antibody to penicillin can be demonstrated in vitro using normal cells if penicillin is in the test system (the antigen being a drug–hapten [erythrocyte] combination) the antibodies to α-methyldopa or pirimicarb cannot. Also in contrast to penicillin, the addition of α-methyldopa or pirimicarb to serum containing the specific antibody does not neutralize their effects. While dogs and humans may react differently to pirimicarb, it cannot be assumed that humans, like the rodents used in the safety evaluation of pirimicarb, will not respond with a haemolytic anaemia, given the susceptible genetic background and exposure. Therefore, the haemolytic anaemia described in a few dogs exposed to pirimicarb is presumed to be relevant.

In a study of chemically-induced autoimmune haemolytic anaemia, groups one male and one female unrelated young adult dogs were fed diets containing pirimicarb (purity, 95%; reference No. not reported) at measured doses of either 25 or 50 mg/kg bw per day. Pirimicarb was suspended in "Dispersol" OG and sprayed onto the morning food ration for each dog. Samples of blood, urine and bone marrow were obtained before exposure and at 2-weekly intervals for the first 12 weeks of the study. Thereafter, the sampling frequency was determined by the clinical and laboratory findings, or by the therapeutic regimen. Male dog No. 146 (receiving a dose of 25 mg/kg bw) and female dog No. 7 (receiving a dose of 50 mg/kg bw) were dosed continuously for at least 110 weeks.

Haematinics were administered to one dog at each dose that showed evidence of anaemia. From week 18, the male at 50 mg/kg bw and from week 24, the female at 25 mg/kg bw were treated with vitamin B12, vitamin B6, iron, pyridoxine and/or folic acid. At weeks 48 and 56 (for the male and female respectively), treatment with all haematinics and dosing with pirimicarb was stopped.

After the results of this phase of the study were obtained, a further investigation was designed to assess the possible involvement of immune mechanisms. Using a chequerboard analysis, the sera of pirimicarb-treated and untreated dogs were analysed for antibody reaction.

In an investigation carried out after their haematological recovery, the two susceptible dogs were dosed again with pirimicarb, the female at 2 mg/kg bw per day between weeks 80 and 86, and the male at 1 mg/kg bw per day between weeks 86 and 92; it was reported that this dog was subsequently dosed at 2 mg/kg bw per day.

The female at 50 mg/kg bw day and the male at 25 mg/kg per day did not become anaemic after continuous dosing for >110 weeks. The results of biochemical, haematological and clinical observations for these dogs were consistently normal.

The male at 50 mg/kg bw per day and the female at 25 mg/kg per day showed clear indications of disturbed erythropoeisis 10 weeks after the start of dosing. Peripheral blood films revealed changes in the size and shape of the erythrocytes and a few nucleated erythrocytes were regularly found. No abnormalities were seen in either the leukocytes or the platelets. When the haemoglobin levels had fallen to 50% of the value measured before exposure, the affected animals were treated with the haematinics listed above.

The two affected dogs were clinically healthy. Falling haemoglobin concentrations were accompanied by reticulocytosis and a marked erythroid hyperplasia, but with no evidence of haemorrhage. Differential cell counts showed a change in the pattern of developing erythrocytes, giving the appearance of transitional megaloblastosis.

Haemolysis was a prominent feature and failure to correct the anaemia with the various haematinics suggested this as the probable cause of the anaemia. Simultaneous withdrawal of pirimicarb and all haematinics was followed by complete haematological recovery. Biochemical tests of liver and kidney function revealed no abnormality. During the period of haematological normality, the biological half-life of the erythrocytes was determined for all the dogs used in this study. The effect of further administration of pirimicarb to the male previously dosed at 50 mg/kg bw per day was to shorten the erythrocyte half-life and produce anaemia.

Genealogical investigation of the four dogs used in this study showed that the male dosed at 50 mg/kg bw per day was the offspring of a bitch that was also the mother of an affected dog used in a previous study. The low incidence of anaemia suggested that sensitivity to pirimicarb might be genetically determined.

Washed erythrocytes from each of the two anaemic dogs were strongly agglutinated by specific anti-gamma globulin serum, while those of the other two dogs were not. Twenty untreated dogs also had negative results in this test. Free antibody was demonstrated in the sera of both anaemic dogs, but not in the sera from either the two unaffected dogs or the 25 untreated dogs.

In an analysis of sera and cells from 20 untreated dogs, no cross-reaction between them was observed. It was shown that the antibody and the antigenicity of the erythrocytes were related in time to the administration of pirimicarb. The antibody was not present in the serum samples before exposure and the erythrocytes possessed the appropriate antigen only when the dogs were receiving pirimicarb. Withdrawal of pirimicarb was followed by a marked decline in antibody titre within 6 weeks and circulating erythrocytes did not react with specific antiglobulin serum. Characterization of the antibody showed it to be an immune type, probably IgG.

The female dog previously dosed at 25 mg/kg bw per day was dosed at 2 mg/kg bw per day for 14 weeks without evidence of a haematological effect. No antibody was detectable in the serum of this animal at the end of this period.

The male dog previously dosed at 50 mg/kg bw per day was dosed at 1 mg/kg bw per day for 12 weeks without evidence of a haematological effect. The antibody had not been completely absent from the serum of this animal, but the titre did not increase, being <1 in 2. It was reported that the dose was then increased to 2 mg/kg bw per day, although the length of the dosing period was not stated. The NOAEL for anaemia in sensitive dogs was 2 mg/kg bw per day (Garner et al., 1995).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

The carcinogenic potential of pirimicarb has been evaluated in three lifetime feeding studies in mice, two in the Alderley Park Swiss-derived mouse and one in the C57 black

mouse. The earliest study (Palmer & Samuels, 1974) was reviewed by the JMPR in 1978, when a request was made to the notifier for another study of carcinogenicity to be undertaken in an appropriate species using a currently (i.e. for 1978) accepted protocol. This study suffered from the high incidence of respiratory disease. Consequently, the Palmer & Samuels (1974) study will not be reviewed here.

Groups of 60 male and 60 female Alderley Park Swiss-derived mice were fed pelletted diets containing pirimicarb (reference Nos BX189 and WED/G/9680; purities, 97.7% and 98.2%, respectively) at a concentration of 0, 0, 200, 400 or 1600 ppm for up to 96 weeks. These concentrations provided doses equivalent to 0, 0, 30, 60 or 240 mg/kg bw per day.

Clinical observations were made, body weights and food consumption were measured and at intervals urine analysis was undertaken to assess the amount of pirimicarb metabolite present. All mice, including any found dead or killed prematurely, were subjected to a full examination post mortem. At the scheduled end of the exposure period, specified tissues were taken for subsequent histopathology and bone-marrow smears were prepared and stored for possible future examination.

The analysis of pirimicarb in rodent diet presented some problems caused by the addition of water to diet before pelleting. These effects were related to a change in the degree of chemical binding of pirimicarb to dietary components and were reduced by a change in diet from expanded Porton rat diet with a vitamin E supplement (PRDE) to Porton combined diet (PCD) (same supplier). No evidence was obtained to show a facile chemical degradation of pirimicarb in diet caused by the pelletting process. Analytical methods were established to adequately monitor the pirimicarb content of diets periodically throughout the study. Availability of dietary pirimicarb was demonstrated by analysis of the hydroxypyrimidine metabolite of pirimicarb in urine (Banham et al., 1980).

The rate of mortality was increased for females at 1600 ppm during weeks 30 to 60 and shortly before termination. None of the other groups showed any differences in rates of mortality compared with the two control groups. There were no specific clinical signs attributable to treatment.

Reduced body-weight gain occurred at intervals throughout the study in male and female mice at 1600 ppm. The main effects were during the first 8 weeks and during weeks 54 to 60 ($p < 0.01$, Student *t*-test). Similar effects on body-weight gain were seen in males at 400 ppm during the first 3 weeks of the study ($p < 0.05$, Student *t*-test).

Food consumption was difficult to measure due to large amounts of wastage, which was consistently damp. These data, therefore, are not fully reliable. Irrespective of these problems, it was apparent that the mice given diets containing pirimicarb at a concentration of 1600 ppm, and to a lesser extent females at 400 ppm, were wasting more food than the others. At 1600 ppm, males had reduced food consumption throughout the study; this was also true for females after week 28. Food utilization was reduced in both sexes at 1600 ppm; this being most apparent during the first 4 weeks, but females continued to show a reduced food utilization over the first 12 weeks. The hydroxypyrimidine metabolite of pirimicarb was detected in all of the urine samples from mice receiving pirimicarb and none was detected in the controls. There was some evidence of a relationship between treatment and the amount of pirimicarb metabolite excreted in females although the correlation was not as good for males.

No treatment-related non-neoplastic effects were found. A number of different types of neoplasms were seen in all groups. In particular, five types occurred at a high frequency: lymphosarcoma, pulmonary tumours (mainly adenomas), hepatic, Harderian gland and pituitary gland tumours. Other types occurred much less frequently. Numbers of tumour-bearing animals per group were similar for males and females, but a number of differences were observed for certain tumour types, particular lymphosarcoma, pulmonary tumours and hepatic nodules. All the tumours seen are common in this strain of mouse and none was unique to mice treated with pirimicarb (Table 29).

There was no evidence of group differences in males in either the incidence or time of occurrence of lymphosarcomas. In females, the incidence of lymphosarcomas in the group receiving 1600 ppm was increased and the latency decreased when compared with the group 1 controls. However, these differences were of the same magnitude when control groups 1 and 2 were compared. Therefore, little significance can be attached to the finding in the group receiving pirimicarb at 1600 ppm.

There was a significant increase in the incidence of pulmonary adenomas in males and females at 1600 ppm. The incidence of such tumours in mice in historical controls has varied from 0% to 28% for males and from 0% to 15.5% for females and illustrates the variability of tumour incidence in the Alderley Park mouse. The incidences of pulmonary adenomas at 1600 ppm were higher than the ranges for historical controls, particularly in females. There was, however, no evidence of development of malignancy in the lungs.

There was a significant increase in liver tumours in male and female mice at 1600 ppm. This finding, however, must be considered against the high and variable values for data from controls, both in the present study and in historical studies. Also, these tumours arose in the absence of any hepatotoxicity lesions in this particular study (i.e. incidences of non-neoplastic lesions in the livers of male and female mice at 1600 ppm were no higher, and often lower, than in the control groups) or in any other study with pirimicarb. However, the diagnostic categories used (and which are no longer used) were type A and type B nodules. Type A nodules include both hyperplastic nodules and benign neoplasms (i.e. preneoplastic nodules constitute a proportion of this category), while type B nodules include

Table 29. Total numbers of selected tumours in mice fed diets containing pirimicarb for 96 weeks

Neoplasm	Dietary concentration (ppm)									
	Males					Females				
	0 (control group 1)	0 (control group 2)	200	400	1600	0 (control group 1)	0 (control group 2)	200	400	1600
Liver										
No. of mice examined	58	59	59	58	57	58	59	57	58	59
Type A nodule	3	9	5	9	15	1	2	3	6	4
Type B nodule	4	6	13	8	17	2	0	3	3	5
Lung										
No. of mice examined	59	60	59	59	58	59	59	59	59	59
Adenoma	9	8	9	8	17	9	4	9	11	18
Carcinoma	0	1	0	0	1	0	1	0	1	0
Pituitary										
No. of mice examined	52	46	45	40	53	51	55	50	52	41
Adenoma	4	6	1	0	0	22	16	16	16	4
Lymphosarcoma	13	15	13	13	14	11	25	18	18	24

From Sotheran et al. (1980)

tumours that show morphological characteristics of malignancy. In males at 1600 ppm, the incidence of both type A and type B nodules were clearly increased compared with that in both control groups. In addition, however, type B nodules were increased in males at 200 ppm, but not at 400 ppm. It is considered, therefore, that these liver tumours provide equivocal evidence for a carcinogenic effect of treatment in this study.

The incidence of pituitary gland adenomas was reduced in males (where, because of the lower incidences in the control group, the reduction was not significant) and in females at 1600 ppm.

The NOAEL for toxicity in mice was 200 ppm, equivalent to 30 mg/kg bw per day, on the basis of reduced body weights at 400 ppm, equivalent to 60 mg/kg bw per day. The NOAEL for carcinogenicity was 400 ppm, equivalent to 60 mg/kg bw per day, on the basis of equivocal evidence for increased incidences of malignant liver tumours in male mice and benign lung tumours in male and female mice at 1600 ppm, equivalent to 240 mg/kg bw per day (Sotheran et al., 1980).

Groups of 55 male and 55 female C57BL/10J$_f$CD-1 Alpk mice were fed diets containing pirimicarb (purity, 97.5%; reference No. P22) at a concentration of 0, 50, 200 or 700 ppm for at least 80 weeks. These concentrations provided doses equal to 0, 6.7, 26.6 or 93.7 mg/kg bw per day for males, respectively, and 0, 9.0, 37.1 or 130.3 mg/kg w/day for females, respectively. Clinical observations, body weights and food consumption were measured and at week 53 blood smears were taken for haematology. All mice, including any found dead or killed prematurely, were subjected to a full examination post mortem. At scheduled termination, cardiac blood samples were taken for haematology, selected organs were weighed and specified tissues taken for subsequent histopathology.

Pirimicarb had no adverse effects on survival and there was no evidence of treatment-related clinical changes. Females given diet containing pirimicarb at a concentration of 700 ppm showed a slight increase in the incidence of eye discharge when compared with that in controls; however, as the incidence was similar to that seen in male controls and treated groups and there was no adverse histopathology, it was considered that this observation was unrelated to treatment with pirimicarb. An increased incidence of subcutaneous masses seen in females at 700 ppm was unrelated to any adverse histopathological changes and was considered to be of no toxicological significance.

At 700 ppm, there was a clear reduction in body weight (by a maximum of 6% and 8% in males and females respectively, $p < 0.01$, Student *t*-test). Food consumption was sporadically reduced in males at 700 ppm, but not in females. There was reduced food utilization efficiency in both sexes at 700 ppm (males, 21%; females, 13%, $p < 0.01$, Student *t*-test) pirimicarb and in females at 200 ppm (8%, $p < 0.05$, Student *t*-test). Other small, but statistically significant, differences from control animals on body weight or food consumption e.g. in males at 50 ppm, were not considered to be toxicologically significant as no effects were apparent at 200 ppm.

At the end of the exposure period, both the erythrocyte counts and the mean cell haemoglobin concentrations were increased, while the mean cell volume and the mean cell haemoglobin were reduced in both sexes at 200 and 700 ppm. There was no evidence of anaemia, no compound-related histopathological changes in bone marrow and no evidence of increased extramedullary haemopoeisis. Decreases in mean cell haemoglobin in males

at 50 ppm were considered to be of no toxicological importance in the absence of changes in other erythrocyte parameters at this dose.

Liver weights were slightly increased in both sexes at 700 or 200 ppm. After adjustment for body weight, the increases were 6% and 9% in males and females 200 ppm, respectively, and 19% and 21% in males and females in the group receiving pirimicarb at a concentration of 700 ppm, respectively ($p < 0.01$). In the absence of any histopathological changes, these increases are considered to be of no toxicological importance. The increased incidence of eye discharge in females at 700 ppm seen at clinical observation was also seen macroscopically and there was also a decreased incidence of uterine distension at this dose.

In males, there was an increase in minimal mononuclear cell infiltration of the renal pelvis at 200 and 700 ppm; however, these increased incidences were similar to the incidences seen in all groups of females, including the controls. In females, there was a slightly increased incidence of minimal lymphoid proliferation in the lung at all doses when compared with controls and an increased incidence of pigmentation of the spleen at 700 ppm.

There was no effect of treatment with pirimicarb on the overall incidence of tumours in mice of either sex. The only neoplastic finding of significance was a small, but statistically significant increase in lung adenomas in females at 700 ppm (Table 30). The incidence, although it was low, was outside the incidence for historical controls (Table 31). A slightly higher, but non-significant incidence of lung adenoma was also observed in males. There was also a small increase the incidence of Harderian gland adenomas in males at 700 ppm and a decrease in the incidence of lymphosarcomas in males of the same group (Rattray, 1998).

It is concluded that oral administration of pirimicarb at a dose of up to 700 ppm for at least 80 weeks produced a small increase in the incidence of benign lung tumours in females, but not in males. The NOAEL for toxicity was 50 ppm, equal to 6.7 mg/kg bw per day, on the basis of slight haematological changes at 200 ppm, equal to 26.6 mg/kg bw per day.

Table 30. Selected neoplastic findings in the lungs of mice fed diets containing pirimicarb for at least 80 weeks

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	50	200	700	0	50	200	700
Adenoma	1	1	1	3	0	0	0	6
Keratinizing squamous epithelioma	0	0	0	0	0	0	0	1

From Rattray (1998)

Table 31. Historical control range of lung adenoma in studies of carcinogenicity in mice

Study duration (No.)	Dates	Range of incidence	
		Males	Females
2-year (15)	March 1984–November 1994	0/50–4/50	0/60–3/55
80-week (6)	May 1994–October 1988	1/50–4/55	0/50–2/55

From Rattray (1998)

Rats

Three studies (which pre-dated the establishment of good laboratory practice (GLP) of carcinogenicity with pirimicarb in rats, in which there were high incidences of respiratory disease, are not reviewed here because they are considered inadequate for risk assessment.

Groups of 64 male and 64 female Alpk:ApfSD rats were fed diets containing pirimicarb (purity, 97.6%; reference No. RS/88/E BXE/583) at a concentration of 0, 75, 250 or 750 ppm for up to 104 weeks. These concentrations provided doses equal to 0, 3.7, 12.3 or 37.3 mg/kg bw per day and 4.7, 15.6 or 47.4 mg/kg bw per day for males and females respectively. Twelve rats of each sex from each group were killed after 52 weeks, while the remainder continued to be exposed to pirimicarb until they were killed after 105 weeks. In addition, satellite groups of 36 male and 36 female rats were fed the same diets; 8 rats of each sex per group were killed after 26, 52 and 78 weeks and 12 rats of each sex per group were killed after 104 weeks, for determination of plasma, erythrocyte and brain cholinesterase activities.

Clinical observations (including ophthalmoscopy), body weights, food consumption, haematology and blood and urine chemistry were measured. At the scheduled times, the rats were killed and subjected to a full examination post mortem. Blood samples were taken at this time, selected organs were weighed and specified tissues were taken for subsequent histopathology.

There was no effect on the incidence of mortality in any of the treated groups and the incidence of clinical abnormalities was generally low, with no evidence for a relationship with treatment. In particular, there were no observations indicative of neurological dysfunction. There were no treatment-related ophthalmological changes.

Body-weight gain was reduced in the group receiving diet containing pirimicarb at a concentration of 750 ppm, such that final body weights were approximately 6% and 13% lower than those of the controls for males and females respectively. At 250 ppm, male body-weight gain was marginally, but statistically significantly reduced during the first 13 weeks of the study and female body-weight gain was slightly reduced throughout the study. Final body weights of the female rats at 250 ppm were approximately 6% lower than those of the controls, while values for male rats were similar to those for the controls. Although there was a marginal reduction in body-weight gain for females at 75 ppm during the second year, this was considered not to be of toxicological significance as it was small, did not form part of a dose–response relationship and values for females in the control group were atypically high during the second year. Furthermore, given the normal body weight progression in rats over a 2-year study, the changes that occur in the first year are more important than those occurring later. At 52 weeks there were statistically significant deficits in body-weight gain in both males (6%) and females (10%) at 750 ppm ($p < 0.01$, Student *t*-test), but not at any lower dose.

Food consumption was generally reduced throughout the study amongst males and females at 750 ppm and there was a marginal reduction amongst females at 250 ppm. There was no effect on males at 250 ppm or on either sex at 75 ppm. Food utilization was generally lower in the groups receiving diet containing pirimicarb at a concentration of 250 and

750 ppm, particularly during weeks 1–4, although at 250 ppm the difference from controls was small.

Haematological analysis showed slight increases in some of the erythrocyte parameters in females in the main group, particularly at week 52. At 750 ppm, these parameters were: haemoglobin concentration, erythrocyte volume fraction, mean cell haemoglobin and mean cell haemoglobin concentration. Both haemoglobin concentration and erythrocyte volume fraction were also increased in females of the main study at week 52 at 250 ppm, and at week 26 at 750 ppm. As similar changes were not seen in the larger, satellite groups of females killed at 52 weeks, and they were largely confined to data obtained for week 52, they are considered to be of no toxicological significance. In males, mean cell volume and mean cell haemoglobin were slightly higher in the group receiving pirimicarb at 750 ppm in weeks 26, 52 and 78 of the main study, but as other erythrocyte parameters were not affected these changes are considered to be incidental to treatment. Several other statistically significant changes were seen, but were all either minor and/or not consistent over a period of time and therefore considered not to be related to treatment.

There were increased levels of plasma cholesterol and triglycerides. Plasma cholesterol concentrations were statistically significantly increased in males in the main study at weeks 13, 26, 52, 78 and 104 at 750 ppm, and at weeks 13 and 26 at 250 ppm, while, in the males killed at week 52, there were no significant increases. In females in the main study, statistically significant increases were observed at weeks 13, 26, 52 and 78 at 750 ppm, at weeks 13 and 26 at 250 ppm and at week 13 at 75 ppm. This finding was supported by an increase in plasma cholesterol in females at 750 mg/kg killed at week 52. Plasma triglyceride concentrations were statistically significantly increased in males in the main study at weeks 52 and 78 in the group receiving pirimicarb at 750 ppm, a result that was supported by increases in plasma triglycerides in males at 750 and 250 ppm killed at week 52. In females in the main study, statistically significant increases were observed at weeks 13 and 26 for the group receiving pirimicarb at 750 ppm and week 26 for the group at 250 ppm, while there were no significant increases in the females killed at week 52. Thus, although there were clear indications of an effect on blood lipids, particularly on cholesterol in males, there were also inconsistencies in the observations that render interpretation difficult. Plasma alkaline phosphatase activities were statistically significantly reduced in males in the main study at weeks 52 and 78 for the group at 750 ppm and at week 13 for the group at 250 ppm, while in the males killed at week 52 the reduction for the group at 750 ppm almost reached statistical significance. In females in the main study, a statistically significant decrease was observed at week 52 for the group at 750 ppm, which was supported by a similar reduction in the females at 750 mg/kg killed at week 52.

Urinary concentrations of protein were increased in males at 750 ppm at week 51 and in all treated males at week 77, but these deviations were not consistent over time and because of the variability of individual urinary concentrations of protein in ageing rats, this is considered to be of no toxicological significance. All other statistically significant differences in urinary parameters were small, inconsistent with time and considered to be of no toxicological significance.

Plasma cholinesterase activities were lower than those of controls for females at 250 ppm (week 26) and at 250 ppm and 750 ppm (weeks 52 and 104) (Table 32). Occasional reductions were also seen in males at these doses, but were with no indication of a

Table 32. Plasma cholinesterase activity(U/l) in rats fed diets containing pirimicarb

Week	Dietary concentration (ppm)							
	Males				Females			
	0 (control)	75	250	750	0 (control)	75	250	750
26	618	599	548*	571	2014	1986	1682*	1745
52	802	777	775	747	2154	2008	1848*	1692**
78	924	848	672**	767*	1851	1891	1808	1748
104	767	952	843	842	2449	2186	2007*	1898**

From Tinston (1992)

* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

dose–response relationship and were therefore considered to be of no toxicological significance. There was no effect on erythrocyte or brain cholinesterase activity, therefore the effects on plasma cholinesterase activities were considered to be evidence only of absorption of pirimicarb.

No treatment-related macroscopic changes were seen at autopsy. In rats killed at 52 weeks, liver weights, adjusted for body weight, were slightly increased in males and females at 750 ppm (12% and 11%, respectively) and in females at 250 ppm (9%), while, at the end of the study, adjusted liver weights were increased in males and females only at 750 ppm (9% and 12%, respectively). No treatment-related changes were seen in any other organ weights.

Male rats fed diet containing pirimicarb at 750 ppm showed small increases in the incidence and severity of necrosis of the brain, vacuolization of the adrenal cortex, minimal renal pelvic transitional cell hyperplasia, renal pelvic vascular ectasia, minimal hepatocellular hypertrophy and altered hepatocytes (clear cell). Males fed at 250 ppm had increased incidences of renal pelvic transitional cell hyperplasia and renal pelvic vascular ectasia. Females showed an increased incidence of slight renal pelvic transitional cell hyperplasia at 250 ppm and 750 ppm. There was also an increased severity of sciatic nerve demyelination and an increased severity and incidence of voluntary muscle degeneration. These changes in the nerve and voluntary muscle were probably related and were considered to represent an exacerbation of a spontaneous age-related change. Increased incidences of findings in a variety of other organs were considered of no toxicological significance.

The incidence of all tumours combined, both benign and malignant, was increased, but the incidence was not statistically significant. There was, however, an overall increased incidence in the number of males with malignant tumours at 250 ppm and 750 ppm, which was statistically significant (Fisher's exact test). This increase was associated with a variety of different malignancies arising in different organs, rather than a single type of tumour and may indicate an increased rate of progression to malignancy. There were also low incidences of astrocytoma of the brain in all treated groups of males and in females at 750 ppm; while these were not statistically significant and no dose-response relationship was evident, none occurred in the controls, so their significance should not be summarily dismissed. The incidences were, for the control group and groups receiving rising doses respectively, in male rats: 0/64, 3/64, 2/64, 3/64 and female rats: 0/64, 1/64, 0/64, 2/64. Data available on controls from 15 studies in this strain of rat in the performing laboratory for 1980–1987 provide a context in which to assess these astrocytomas. In male rats there were 15 astrocytomas

among 542 controls (mean, 2.76%; maximum, 4.83%). In female rats there were 8 astrocytomas among 542 controls (mean, 1.48%; maximum, 3.85%). Thus, the highest incidences in the current study (males, 3/64, 4.69%; females, 2/64, 2.13%) lie just within the upper limits of the ranges for historical controls. Nevertheless, the occurrence of relatively high incidences in the males and females of treated groups within one experiment is a matter of some concern when no astrocytomas were diagnosed in either males or females of the concurrent control groups.

The NOAEL was 75 ppm, equal to a mean dose of 3.7 mg/kg bw per day, on the basis of reductions in body weight and increases in plasma cholesterol and triglycerides at 250 ppm, equal to 12.3 mg/kg bw per day (Tinston, 1992).

Overall, the Meeting concluded that pirimicarb is unlikely to pose a carcinogenic risk to man.

2.4 Genotoxicity

Pirimicarb was tested for genotoxicity in a range of assays, both in vitro and in vivo (Table 33). There was no evidence of genotoxicity in vitro in tests for gene mutation in two studies in bacteria (*S. typhimurium* and *E. coli*) (Trueman, 1980; Callander, 1995). There was, however, a significant positive response in one study for mutations in mouse lymphoma L5178Y cells in vitro, using the currently recommended microwell version of this assay (Clay, 1996). There were increases in the numbers of both small and large mutant colonies at concentrations that permitted high levels of survival. This distinction is made because the assay is believed to be able to detect both gene mutations (manifest as large colonies) and large regions of damage, including small deletions and microscopically visible chromosomal aberrations (manifest as small colonies, because they grow more slowly).

In a single study for chromosomal aberrations in cultures of human lymphocytes taken from two donors, no significant increases in the proportion of abnormal cells were observed

Table 33. Studies of genotoxicity with pirimicarb

End-point	Test object	Dose (LED/HID)	Purity (%)	Result	Reference
In vitro					
Gene mutation	*S. typhimurium* strains TA100, TA1535, TA1537, TA1538 TA98 (±S9)	2500 µg/plate	Approx. 98	Negative[b]	Trueman (1980)
Gene mutation	S. typhimurium strains TA100, TA1535, TA1537, TA98; *E.coli* WP2, WP2*uvrA* (±S9)	5000 µg/plate	97.6	Negative[b]	Callander (1995)
Gene mutation	Mouse lymphoma L5178Y cells, Tk^+ locus (±S9)	1400 µg/ml –S9 100 µg/ml +S9	97.5	Negative Positive	Clay (1996)
Chromosomal aberration	Human lymphocytes cells (±S9)	500 µg/ml	98.2	Negative	Wildgoose et al. (1987)
In vivo					
Unscheduled DNA synthesis in vivo/in vitro	Liver cells from male Alpk:APfSD rats, 4 h and 12 h after dosing	200 mg/kg bw per os, single dose	98.3	Negative	Kennelly (1990)
Micronucleus formation	Bone-marrow cells from male C57BL/6JfCD-1/Alpk mice, 24 h, 48 h and 72 h after dosing	69.3 mg/kg bw, per os, single dose	97.3	Negative	Jones & Howard (1989)
Dominant lethal mutation	Male CD1 mice, 1–8 weeks after dosing	20 mg/kg bw, per os, five doses	Not reported	Negative	McGregor (1974)

LED, lowest effective dose; HID, highest ineffective dose; S9, 9000 × *g* supernatant from rodent liver

after incubation exposure for 3 h in an experiment conducted in the presence and absence of an exogenous activation system (Wildgoose et al., 1987).

No genotoxic activity was observed in three experiments conducted in vivo: an assay for unscheduled DNA synthesis in rat liver cells, an assay for micronucleus formation in bone marrow and an assay for dominant lethal effects in mice.

For the study of unscheduled DNA synthesis in vivo/in vitro in liver, groups of male Alpk:APfSD rats were killed either 4 h or 12 h after the administration of pirimicarb as a single oral dose at 50, 100 or 200 mg/kg bw (4 h) or 50 or 100 mg/kg bw (12 h), by gavage. A vehicle (corn oil, 10 ml/kg bw) control and a positive control (6-*p*-dimethylaminophenylazobenzthiazole, 40 mg/kg bw) group of four rats were dosed and subgroups of two rats per group were killed at 4 h and 12 h. Pirimicarb did not induce unscheduled DNA synthesis at any dose or time-point, while the positive control induced substantial DNA repair (Kennelly, 1990).

In the test for micronucleus induction in mouse bone-marrow cells, groups of 15 male and 15 female C57BL/6JfCD-1/Alpk mice were given pirimicarb (technical) at a dose of 0, 43.3 or 69.3 mg/kg bw orally by gavage on a single occasion, these doses being 50% and 80% of the median lethal dose. A group of five males and five female was given the positive control substance cyclophosphamide as an oral dose at 65 mg/kg bw. Subgroups of five males and five females per group were killed approximately 24, 48 and 72 h after treatment (except for animals in the positive control group, which were killed after 24 h). No increases in micronucleated polychromatic erythrocytes were observed in either sex in any group dosed with pirimicarb. Large increases in the incidence of micronucleus formation were observed in the positive control group (Jones & Howard, 1989).

In the dominant lethal effects experiment, groups of 15 male CD-1 mice of proven fertility were dosed with pirimicarb at a dose of 0, 10 or 20 mg/kg bw per day orally by gavage for 5 days. Positive control groups of a similar size were dosed with ethylmethanesulfonate, either intraperitoneally at a dose of 150 mg/kg bw on a single occasion, or orally at 100 mg/kg bw per day on 5 days. The males were then mated with fresh pairs of virgin females at weekly intervals for 8 weeks and the females were killed 15 or 16 days after the beginning of each mating period. They were assessed for dominant lethal effects; no effects were found in the groups treated with pirimicarb, while the positive control groups showed substantial effects in mating weeks 1 and 2, particularly in the group treated with multiple oral doses (McGregor, 1974).

Thus, the single indication of mutagenic activity observed in mouse lymphoma L5178Y cells in vitro was not supported by other assays in vitro or by results from assays in vivo. The Meeting concluded that pirimicarb is unlikely to be genotoxic.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

Groups of 26 male and 26 female weanling Alpk:ApfSD rats (F_0 generation) were fed diets containing pirimicarb (purity, 97.3%; reference No. RS/88/E) at a concentration of 0, 50, 200 or 750 ppm, equal to 0, 5.41, 21.7 or 81.8 mg/kg bw per day for males and 0, 5.64,

22.5 or 83.5 mg/kg bw per day for females, averaged over the first 10 weeks of exposure in a two-generation study of reproduction. After 10 weeks, the rats were mated and allowed to rear the ensuing F_{1a} litters to weaning. The breeding programme was repeated with the F_1 parents (selected from the F_{1a} offspring) after 10 weeks of exposure. The dietary exposures for the F_1 parental generation were equal to 0, 5.76, 23.2 or 90.1 mg/kg bw per day for males and 0, 6.04, 24.3 or 96.3 mg/kg bw per day for females, averaged over the first 10 weeks of exposure. The ensuing F_{2a} litters were reared to weaning. The diets containing pirimicarb were fed continuously throughout the study. A gross pathological examination was carried out on all parents and selected offspring. Histopathological examination was restricted to abnormalities and reproductive tract from animals suspected to be infertile.

In the F_0 generation, there were no adverse effects of pirimicarb on survival or evidence of treatment-related clinical changes. Administration of pirimicarb at 750 ppm was associated with reduced body-weight gain. Mean body weights at the end of the pre-mating period were lower than control values by 5% and 7% for F_0 males and females respectively (Table 34). As a result of the differences in starting body weights, the mean body weights of the F_1 animals at the end of the pre-mating period were lower by 12% and 11% respectively for males and females fed pirimicarb at 750 ppm compared with controls. The reduced body-weight gain in females fed at 750 ppm persisted throughout gestation and lactation in both generations, although a lesser effect was seen for the F_1 females. There were no adverse effects on body-weight gain in animals fed at 200 or 50 ppm.

Food consumption and food utilization were reduced at 750 ppm during the pre-mating period, particularly during weeks 1–4, as well as during gestation, but not during lactation. There were no adverse effects on food consumption at 200 or 50 ppm.

Table 34. Body weight (g) of rats fed diets containing pirimicarb in a multigeneration study

Generation/time	Dietary concentration (ppm)							
	Males				Females			
	0	50	200	750	0	50	200	750
F_0 parents								
Pre-mating:								
Initial weight	84.2	83.8	83.1	84.5	77.1	79.9	78.4	77.5
Final weight	434.5	443.6	435.0	414.9*	259.0	262.4	253.0	240.5**
Pregnancy:								
Initial weight	—	—	—	—	261.8	265.2	262.3	243.0**
Final weight	—	—	—	—	380.2	384.3	378.0	354.9**
Lactation:								
Initial weight	—	—	—	—	291.4	292.1	285.5	272.7**
Final weight	—	—	—	—	331.6	331.9	333.9	304.6**
F_1 parents								
Pre-mating:								
Initial weight	76.3	77.0	74.6	68.2	70.3	71.7	70.8	65.2
Final weight	440.7	442.7	437.9	386.2**	251.6	257.0	244.4	224.7**
Pregnancy:								
Initial weight	—	—	—	—	263.3	266.1	255.3	233.5**
Final weight	—	—	—	—	389.8	390.6	380.3	348.2**
Lactation:								
Initial weight	—	—	—	—	298.5	308.8	297.1	274.2**
Final weight	—	—	—	—	340.5	342.8	331.3	304.9**

From Moxon (1991)

* Statistically significant difference from control group mean, $p < 0.05$ (Student *t*-test, two-sided)

** Statistically significant difference from control group mean, $p < 0.01$ (Student *t*-test, two-sided)

There was no evidence of an adverse effect of pirimicarb on fertility or general reproductive performance at any of the doses tested. There was no effect of pirimicarb on litter size at birth or pup survival. Mean pup birth weight, per sex, was slightly lower in the group receiving pirimicarb at 750 ppm than in the controls, particularly in the F_{2a} litters. There was a subsequent effect on body weight at 750 ppm in both the F_{1a} and F_{2a} offspring, with body weights at day 29 being reduced by 7% and 8% for F_{1a} males and females and by 15% and 10% for F_{2a} males and females respectively. There was no effect on body weights of offspring at 50 or 200 ppm.

There were no compound-related macroscopic or microscopic findings in parents or offspring in either generation. There was no effect on testis weight in either generation. The NOAEL for reproductive parameters was 750 ppm, equal to 88 mg/kg bw per day (calculated from the pre-mating periods of the F_0 and F_1 parents), the highest dose tested. The NOAEL for toxicity in adult rats was 200 ppm, equal to 22.9 mg/kg bw per day (calculated from the pre-mating periods of the F_0 and F_1 parents), on the basis of reduced body-weight gain at 750 ppm, equal to 88 mg/kg bw per day. The NOAEL for offspring toxicity was 200 ppm, on the basis of reduced pup-weight gain at 750 ppm. There were no effects on reproductive indices at doses of up to 750 ppm, the highest dose tested (Moxon, 1991). The Meeting concluded that pirimicarb is not a reproductive toxicant in rats.

(b) Developmental toxicity

Rats

In a study of developmental toxicity, groups of 24 time-mated, female Alpk:ApfSD rats were given primicarb (purity, 97.3%; reference No. RS/88/E/BXE583) at a dose of 0, 0, 10, 25 or 75 mg/kg bw per day in corn oil by gavage on days 7 to 16 of gestation. The day of confirmation of mating (when spermatozoa were detected) was designated day 1 of gestation. The control group of animals received corn oil only. On day 22 of gestation, the females were killed and their uteri examined for live fetuses and intrauterine deaths. The fetuses were weighed, examined for external/visceral abnormalities, sexed, eviscerated and stained for skeletal examination.

One pregnant rat at 75 mg/kg bw per day was killed on day 7 of gestation after a dosing accident. All other rats survived for the duration of the study. Clinical observations in the dams were of low incidence or not dose-related and of the type commonly seen in the strain of rat used. Body-weight gain was lower in rats at 75 mg/kg bw per day during the pre-dosing period (25%) and there was a statistically significant decrease in body-weight gain compared with that of controls throughout the dosing period in this group (26% during days 7 to 16 of gestation). The body-weight gains in all treated groups were similar to those of rats in the control group during the post-dosing period, but overall weight gain during gestation remained statistically significantly lower for dams at 75 mg/kg bw per day. There was a statistically significant reduction in food consumption for rats receiving pirimicarb at a dose of 75 mg/kg bw per day, compared with that of controls during the dosing period (days 7 to 16 of gestation). Food consumption for all treated groups was similar to that of controls during the post-dosing period. Macroscopic changes seen post mortem were of a type and incidence commonly seen in the strain of rat used in this study and were considered not to be related to treatment.

Administration of pirimicarb at 75 mg/kg bw per day produced a significant reduction in fetal weight (8%, $p < 0.01$), with associated small reductions in litter weight and gravid

uterus weight. There was no evidence of any difference in the number or survival of the fetuses in utero at any dose, when compared with control values.

Major defects were seen in four fetuses in this study; 1, 1, 0 and 2 occurring in the groups at 0, 10, 25 and 75 mg/kg bw per day, respectively. These were: extreme dilation of the left ureter; umbilical hernia; fusion of arches of the second and third lumbar vertebrae; and a sixth digit on a hind paw. The defects were clearly unrelated and of low incidence and therefore considered to be spontaneous and not a result of treatment with pirimicarb.

The overall proportion of fetuses with minor external or visceral soft tissue defects was not statistically significantly increased by treatment with pirimicarb. Overall, there was a clear statistically significant increase in the incidence of fetuses with minor skeletal defects at 75 mg/kg bw per day. The incidences of fetuses with minor skeletal defects were: 50/265 (19.5%), 70/287 (24.5%), 65/270 (25.5%) and 92/244 (36.3%, $p < 0.01$) at 0, 10, 25 and 75 mg/kg bw per day, respectively. Almost all fetuses in all groups had some kind of skeletal variation, although the incidence was significantly higher at 75 mg/kg bw per day: 255/265 (96.5%), 276/287 (95.8%), 262/270 (97.3%) and 243/244 (99.6%, $p < 0.01$). These minor defects and variants were mainly incomplete ossification of cervical centra, strenebrae, odontoid and calcaneum, but also frequent in all groups were full ossification of the transverse processes of the fourth lumbar vertebra and bipartite fifth strenebrae.

The occurrence of an unossified centrum of the third cervical vertebra (minor defect) was statistically significantly increased at 75 mg/kg bw per day and was slightly increased at 10 mg/kg bw per day. However, the lack of effect at 25 mg/kg bw per day and the fact that 8 out of 23 affected fetuses were from one litter in the group at the lowest dose suggests that the increase seen at this dose is of no toxicological significance. The incidences in all test groups were within the ranges for historical controls. The incidence of unossified second cervical vertebrae (a variant) also became non-significant at 10 mg/kg bw per day after exclusion of this single litter.

There were statistically significant increases in the incidence of bipartite fifth sternebrae (minor defect) at 25 and 75 mg/kg bw per day. The incidence at 25 mg/kg bw per day was just outside the range for historical controls. A bipartite fifth sternebra is a slightly more retarded form of a partially ossified fifth sternebra and therefore these two findings should be considered together for interpretative purposes. Even when combined, however, there was no statistically significant differences between the control group and the group receiving pirimicarb at a dose of 25 mg/kg bw per day.

Only two external/visceral variants (slightly dilated and kinked ureter) were recorded. The incidence of the latter at 10 mg/kg bw per day was statistically significantly higher than that of the controls, but was within expected ranges when compared with historical data. In the absence of a dose–response relationship, this small increase was considered to be unrelated to treatment.

Most fetuses at all doses had one or more skeletal variants. Five types of skeletal variant showed statistically significant differences in one or more treated groups. The incidence of unossified odontoid was statistically significantly increased in all treatment groups with the highest incidence occurring at 75 mg/kg bw per day; however, as no dose–response relationship was apparent at the lower doses, the magnitude of the differences was small compared with the values for controls and the values fell within the range for recent

historical controls, the increased incidence at these doses is considered not to be of toxicological significance. The incidences were: 43/265 (16.2%), 76/287 (26.5%, $p < 0.01$), 60/270 (22.2%, $p < 0.05$) and 101/244 (41.4%, $p < 0.01$) at 0, 10, 25 and 75 mg/kg bw per day, respectively.

The incidence of unossified centrum of the second cervical vertebra was significantly increased at 75 mg/kg bw per day and was slightly increased at 10 mg/kg bw per day, but was within the range for historical controls.

There was a statistically significant increase in the incidence of fully ossified transverse processes of the fourth lumbar vertebra, unossified calcaneum and partially ossified fifth sternebra at 75 mg/kg bw per day compared with controls. The increased incidence of fully ossified transverse processes of the fourth lumbar vertebra represents a slight increase in ossification, in contrast to the other observations that are suggestive of a delay in ossification.

There was a statistically significant increase compared with control values in the degree of reduced ossification of the *manus* (scored on a six-point scale) at 75 mg/kg bw per day, but no effects were seen at lower doses: 2.92, 3.06, 2.94 and 3.14 ($p < 0.05$) at 0, 10, 25 and 75 mg/kg bw per day, respectively.

There was no evidence that pirimicarb is teratogenic to the rat at any of the doses tested in this study. Administration of pirimicarb at a dose of 75 mg/kg bw per day resulted in maternal toxicity and fetotoxicity as demonstrated by reductions in fetal weight, litter weight and gravid uterus weight. There was evidence of poorer ossification at this dose. Administration of pirimicarb at a dose of 25 mg/kg bw per day did not result in any maternal or fetal toxicity and is considered to be the no-effect level, although there were sporadic increases in some variants at this dose and at 10 mg/kg bw per day. The NOAELs in the study of developmental toxicity in rats were 25 mg/kg bw perday for the dams, on the basis of decreases in food consumption and body-weight gains at 75 mg/kg bw per day, and 25 mg/kg bw per day for the fetuses, on the basis of increased incidences of minor defects and variants at 75 mg/kg bw per day (Hodge, 1989).

The Meeting concluded that pirimicarb is neither a teratogen nor a developmental toxicant in rats.

Rabbits

In a study of developmental toxicity, groups of 20 artificially inseminated, female New Zealand White rabbits were given pirimicarb (purity, 97.3%; reference No. RS/88/E/BXE583) at a dose of 0 (19 animals), 2, 10 or 60 mg/kg bw per day by gavage in corn oil on days 7 to 19 of gestation inclusive. The day of artificial insemination was designated day 1 of gestation. On day 30 of gestation, the females were killed and their uteri examined for live fetuses and intrauterine deaths. The fetuses were weighed, examined for external and visceral abnormalities, sexed, eviscerated and stained for skeletal examination.

Two does at 60 mg/kg bw per day were killed during gestation for humane reasons. The clinical observations recorded for these rabbits before their death and the findings recorded at examination post mortem were non-specific and considered not attributable to

pirimicarb as the remaining animals in this group did not show similar signs. Administration of pirimicarb at a dose of 60 mg/kg bw per day resulted in a marked reduction in body-weight gain during the dosing period (days 7 to 19). This reduction was mainly confined to the first 3 days of dosing and there was some indication of weight recovery after the dosing period. There were no significant difference from the controls in body-weight gain at 10 mg/kg bw per day and the significant reductions that occurred at 2 mg/kg bw per day were not clearly not related to treatment. There was a reduction in food consumption in the groups receiving pirimicarb at a dose of 10 or 60 mg/kg bw per day during the dosing period. Differences in food consumption at 10 mg/kg bw per day were considered to reflect pre-dosing differences relative to the control group in which two animals had high food consumption.

No treatment-related changes were observed at any dose at examination post mortem. The incidence of cysts in the fatty tissue surrounding the ovaries (0, 3, 4 and 5, in the control group, and at 2, 10 and 60 mg/kg bw per day respectively) was within the incidence for historical controls.

There was no evidence of any adverse effects on the number, growth or survival of the fetuses in utero caused by treatment with pirimicarb at any dose. The incidences of fetuses with major defects were 5, 3, 2 and 5 in the groups receiving pirimicarb at a dose of 0, 2, 10 or 60 mg/kg bw per day respectively. The defects were of various types and occurred across all of the groups. There were a number of major defects in the treated groups that were not seen in the concurrent control group, but which have been seen previously in control or treated animals in the laboratory in which the study was performed. A gross malformation of the pinna was seen, however, in one fetus at 60 mg/kg bw per day and this had not been recorded previously in the laboratory laboratory in which the study was performed. In the absence of any dose-related trends, these isolated defects are considered to be spontaneous occurrences and not related to treatment with pirimicarb. There was no effect on the incidence of external/skeletal minor defects or variants.

The maternal NOAEL in this study of developmental toxicity in rabbits was 10 mg/kg bw per day on the basis of reductions in body-weight gain at 60 mg/kg bw per day, and the NOAEL for developmental toxicity was 60 mg/kg bw per day, the highest dose tested (Milburn et al., 1989).

The Meeting concluded that pirimicarb is not teratogenic and does not present a developmental hazard in rabbits.

2.6 Special studies

(a) Delayed neurotoxicity

No studies of delayed neurotoxicity with pirimicarb were available, it being argued that, because it is a carbamate insecticide and not an organophosphorus compound, such studies are not necessary. The insecticidal carbamates have shown no evidence of an ability to induce a delayed neurotoxic syndrome similar to that seen with some organophosphorus compounds. Ina review of 17 carbamates administered as single doses at doses ranging from half to several times the LD_{50}, no behavioural evidence of neurotoxicity was observed during observation periods of 22 days, and no degeneration of the sciatic nerve was seen at autopsy (Baron, 1991).

(b) Neurotoxic potential

Groups of 15 male and 15 female Alpk:AP_fSD (Wistar-derived) rats were given technical-grade pirimicarb (purity, 97.6% w/w; batch No. P16) as single, oral doses at 0, 10, 40 or 110 mg/kg bw by gavage. Ten rats of each sex per group were allocated to the main study and were killed 2 weeks later. The remaining five rats of each sex per group were included as a satellite group for the purpose of measuring brain, erythrocyte and plasma cholinesterase activities, and brain neuropathy target esterase activity, on day 1.

All rats were observed before exposure and daily throughout the study for any changes in clinical condition. In addition, detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, were performed during week −1, and on days 1, 8 and 15. Locomotor activity was also monitored during week −1, and on days 1, 8 and 15. Body weights and food consumption were measured on occasions throughout the study. At the end of the scheduled period, five rats of each sex per group were killed and subjected to a full examination post mortem. Brain weight, length and width were recorded for these animals. Blood samples were taken and the brain was removed from selected animals for determination of brain, erythrocyte and plasma cholinesterase activities, and brain neuropathy target esterase activity. Selected nervous system tissues were removed, processed and examined microscopically.

One female that received pirimicarb at a dose of 40 mg/kg bw and two males and two females that received a dose of 110 mg/kg bw died or were killed for humane reasons after dosing on day 1. One other female that received pirimicarb at a dose of 10 mg/kg bw was killed for humane reasons on day 1; however, as the clinical signs and macroscopic findings seen were consistent with a possible dosing accident, the demise of this animal could not be attributed to administration of pirimicarb. A similar conclusion cannot be reached in the other cases.

Transient, treatment-related clinical signs were seen on day 1 for the majority of animals at 110 mg/kg bw, and for a few females at 10 or 40 mg/kg bw, with full recovery usually occurring by the following day. There was no adverse effect on body weight, although food consumption was slightly, but significantly, reduced during week 1 for males at 40 or 110 mg/kg bw.

Motor activity was significantly reduced in males at 40 and 110 mg/kg bw 6–10 min after dosing on day 1. Slightly lower landing foot splay and slightly increased time to tail flick were seen on day 1 for females at 110 mg/kg bw, but not for males. Locomotor activity was also slightly reduced for females at 40 or 110 mg/kg bw on day 1. On days 8 and 15, however, landing foot splay, time to tail flick and locomotor activity for these rats were normal.

Inhibition of brain cholinesterase activity of (method based on that of Ellman et al., 1961, but not mentioned in the report) was apparent in both sexes after dosing with pirimicarb at 110 mg/kg bw on day 1 and was considered to be indicative of neurotoxic potential (males: control, 12.62 IU/g; 110 mg/kg bw, 9.71 IU/g; $p < 0.01$; females: control, 12.04 IU/g; 110 mg/kg bw, 9.68 IU/g; $p < 0.05$), but not on day 15.

Reductions in erythrocyte (15%) and plasma (45–51%) cholinesterase activity in rats at 110 mg/kg bw, and reductions in plasma cholinesterase activity for rats at 10 or 40 mg/kg bw, were also seen on day 1, but not on day 15.

There was no evidence among rats surviving to termination of any treatment-related effects on brain weight, length or width and no macroscopic findings that could be attributable to treatment with pirimicarb. Comprehensive histopathological evaluation revealed no treatment-related changes in the central or peripheral nervous system of animals that received pirimicarb at a dose of 110 mg/kg bw.

At 110 mg/kg bw, there were clear cholinergic signs is some animals (whether they occurred in the same few animals was not specified), especially in females (not uncommon with carbamates and organophosphates), without a clear correlation with a toxicological significant inhibition of brain or erythrocytic acetylcholinesterase activity. It is likely that this was attributable to the usual technical problems associated with this assay in animals treated with carbamates (i.e. quick spontaneous reactivation in vivo and in vitro during preparation of the sample for the assay. It is not possible from the report to ascertain whether greater inhibition of cholinesterase activity occurred in symptomatic animals. It is difficult to discard the data obtained at 40 mg/kg bw as not significant, while those (very few) at 10 mg/kg bw might be considered incidental. Observations and tissue sampling were performed 3 h after dosing, which was reported to be the time of peak effect (presumably inhibition of acetylcholinesterase activity); however, with carbamates, differences in sampling and measurement time of 0.5 h can be relevant. In conclusion, the NOAEL for neurotoxic potential in rats given a single dose of pirimicarb was 10 mg/kg bw. This evaluation was based on mortalities, adverse clinical signs and effects on landing foot splay, time to tail flick and locomotor activity on day 1, reductions in food consumption during week 1 and significant inhibition of brain, erythrocyte and plasma cholinesterase activities at 110 mg/kg bw and reduced food consumption, transient clinical signs, reduced motor activity and inhibition of plasma cholinesterase activity at 40 mg/kg bw. It is recognized that the effect is C_{max}-dependent, and reversible (Horner, 1996a).

Groups of 12 male and 12 female Alpk:AP$_f$SD (Wistar-derived) rats were given diets containing technical-grade pirimicarb (purity, 97.6% w/w; batch No. P16) at a concentration of 0, 75, 250 or 1000 ppm for 90 days. These concentrations were equal to 0, 5.6, 19.2 and 77.1 mg/kg bw for males and 0, 6.6, 21.8 and 84.4 mg/kg bw for females.

All rats were observed before exposure and daily throughout the study for any changes in clinical condition. In addition, detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness, were performed in weeks −1, 5, 9, and 14. Locomotor activity was also monitored in weeks −1, 5, 9 and 14. Body weights and food consumption were measured weekly throughout the study, and food utilization was calculated. At the end of the scheduled period, six rats of each sex per group were killed and subjected to a full examination post mortem. Brain weight, length and width were recorded and selected nervous system tissues were removed from these animals, processed and examined microscopically. Blood samples were taken from the remaining six rats of each sex per group at termination for determination of plasma and erythrocyte cholinesterase activities, and the brains of these animals were removed and submitted for determination of brain cholinesterase and neuropathy target esterase activities.

Throughout the study, there were no clinical signs that could be attributed to the administration of pirimicarb. At dietary concentrations of 250 or 1000 ppm, there were treatment-related effects on growth associated with reductions in food consumption and/or food utilization. Group mean body weight, adjusted for initial weight, for males and females at 1000 ppm was statistically significantly lower than that of concurrent controls throughout

weeks 2 to 14. At week 14, group mean adjusted body weight values for males and females at this dose were 91.5% and 92.1% of those for concurrent controls, respectively. For rats at 250 ppm, group mean body weight, adjusted for initial weight, was statistically significantly lower than that of concurrent controls throughout weeks 7 to 14 for males and during weeks 9 and 12–14 for females. At week 14, group mean adjusted body weight values for males and females at this dose were 94.1% and 95.1% that of concurrent controls, respectively.

Group mean food consumption for males and females at 1000 ppm was slightly lower than that of concurrent controls throughout the study, with these differences often attaining statistical significance for females. Group mean food utilization was statistically significantly lower than that of concurrent controls for males at 250 or 1000 ppm during weeks 1–4 and 5–8. Food utilization for females at 250 or 1000 ppm was also slightly lower than that of controls during weeks 1–4, although these differences did not attain statistical significance. Slightly reduced food utilization was also seen for males at 75 ppm during weeks 5–8. However, in the absence of any effects on food utilization during weeks 1–4, and as no effects on body weight and food consumption were apparent for these animals throughout the study, this was considered to be of no toxicological significance.

During the functional observation batteries (FOB), there were no clinical signs that could be attributed to administration of pirimicarb. In addition there were no changes in any of the quantitative or qualitative components of the FOB or effects on locomotor activity that could be attributed to administration of pirimicarb.

At autopsy, there were no treatment-related effects on brain weight, length or width, on brain, erythrocyte and plasma cholinesterase activities or on neuropathy target esterase activity. Comprehensive histopathological evaluation of the nervous system revealed no evidence of any treatment-related changes. The NOAEL was 75 ppm, equal to 5.6 mg/kg bw per day, on the basis of reductions in body weight at 250 ppm, equal to 19.2 mg/kg bw per day. The NOAEL for neurotoxic potential, after dietary administration of pirimicarb for 90 consecutive days, was 1000 ppm, equal to 77.1 mg/kg bw per day, the highest dose tested (Horner, 1996b).

(c) Other toxicological studies

(i) Studies of toxicity with metabolites of pirimicarb

Studies of toxicity have been conducted on a number of metabolites of pirimicarb from three different classes: the carbamate metabolites, the hydroxypyrimidine metabolites, and the guanidine metabolites. The International Union of Pure and Applied Chemistry (IUPAC) names, code names and occurrence of the metabolites are detailed in Appendix 1.

Acute toxicity of metabolites

The acute oral toxicities of pirimicarb hydroxypirimidine metabolite 2-amino-5,6-dimethylpyrimidin-4-ol (R31680; 062/14) and carbamate metabolite 2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (R35140; 062/03, reference No. and purity not given) were evaluated in groups of six fasted female Alderley Park SPF Albino rats given either 2-amino-5,6-dimethylpyrimidin-4-ol (R31680) at a dose of 2500 mg/kg bw (apparently the only dose) or 2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (R35140) at a dose of 50, 64, 80, 100, 500 or 1000 mg/kg bw by gavage in Tween 80. The rats were

observed for 14 days after dosing. After dosing with 2-amino-5,6-dimethylpyrimidin-4-ol (R31680), some of the animals became subdued and showed slight urinary incontinence for a few days, but no deaths occurred. In rats dosed with 2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (R35140), toxic signs typical of cholinesterase inhibition (muscular fibrillation, salivation, urinary incontinence and chromodacryorrhoea) were observed. All surviving animals had fully recovered within 3 days. The LD_{50} for 2-amino-5,6-dimethylpyrimidin-4-ol (R31680) was >2500 mg/kg bw, which was thus significantly less toxic than pirimicarb, while the LD_{50} for 2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate (R35140) was calculated to be 79 mg/kg bw (95% CI, 72–88) in female rats, which is comparable with the LD_{50} of pirimicarb itself in fasted rats (Parkinson, 1974).

The acute oral toxicities of pirimicarb hydroxypirimidine metabolites 2-dimethylamino-5,6-dimethyl pyrimidin-4-ol (R31805) and 5,6-dimethyl-2-(methylamino)pyimidin-4-ol (R34865; 062/07), both of which are plant and mammalian metabolites, and carbamate metabolites desmethylformamido pirimicarb (R34885; 062/04), desmethyl pirimicarb (R34836; 836/01), both of which are plant metabolites (reference No. and purity not given), were evaluated in groups of three fasted female Alderley Park SPF albino rats treated at dose ranges that varied for each substance given by gavage in propylene glycol. The rats were observed for 14 days after dosing.

The acute LD_{50}s values were: desmethylformamido pirimicarb (R34885), 50–100 mg/kg bw; desmethyl pirimicarb (R34836), 200–400 mg/kg bw; 2-dimethylamino-5,6-dimethyl pyrimidin-4-ol (R31805), 800–1600 mg/kg bw; and 5,6-dimethyl-2-(methylamino)pyimidin-4-ol (R34865), 2000–2500 mg/kg bw.

For desmethylformamido pirimicarb (R34885) and 5,6-dimethyl-2-(methylamino) pyimidin-4-ol (R34865), no specific signs of toxicity were observed, while for desmethyl pirimicarb (R34836) and 2-dimethylamino-5,6-dimethyl pyrimidin-4-ol (R31805), observations were made that were suggestive of the inhibition of cholinesterase activity. Only desmethylformamido pirimicarb (R34885) showed toxicity comparable with that of pirimicarb itself; the other three metabolites were less toxic than pirimicarb (Lefevre & Parkinson, 1989).

The acute oral toxicities of pirimicarb guanidine metabolites guanidine hydrochloride, methyl guanidine sulfate and dimethyl guanidine hydrochloride (batch Nos and purities not given) were evaluated in groups of six fasted female Alderley Park SPF albino rats treated with these substances at dose ranges that varied for each metabolite, by gavage in water. The rats were observed for 14 days after dosing. The signs of toxicity observed were subdued behaviour, piloerection and urinary incontinence. These signs were no longer evident after 5–7 days, The LD_{50} values were: guanidine hydrochloride, 1105 mg/kg bw (95% CI, 1000–1220 mg/kg bw); methyl guanidine sulfate, 1105 mg/kg bw (95% CI, 950–1285 mg/kg bw); dimethyl guanidine hydrochloride, 1445 mg/kg bw (95% CI, 1215–1720 mg/kg bw). All three compounds were thus appreciably less toxic than pirimicarb itself (Parkinson, 1975).

Repeated doses of metabolites

The plant carbamate metabolite desmethylpirimicarb (purity, >98% w/w; R34836; batch No. R34836P4) was studied for its general toxic and haematological effects and its effects on cholinesterase inhibition in Alderley Park SPF albino rats treated with repeated

doses. The study consisted of two parts. For the toxicity and haematological investigation, groups of 20 male and 20 female rats were given desmethylpirimicarb (R34836) at a dose of 25 or 100 mg/kg bw per day by gavage. A control group of 10 males and 10 females was given the vehicle only ("Lissatan" AC). Half the animals at each doses were given 14 consecutive daily doses and the remainder were given 28 consecutive daily doses. General observations, body weight, blood and urine biochemistry and haematological parameters were measured. At the end of the study, all the rats were examined post mortem. Selected tissues were examined histopathologically.

For the assessment of cholinesterase inhibition, groups of five rats of each sex per group were given R34836 at a dose of 0, 1.5, 5, 25 or 100 mg/kg bw per day for 28 days. Blood samples were taken from all rats before dosing, 1 h after the 14th dose and 1 h after the 28th dose for measurement of erythrocyte and plasma cholinesterase activity. Brain cholinesterase activity was measured at autopsy.

In the first part of the study, one rat at 25 mg/kg was found dead after 10 doses, while none of the other rats in this group showed any clinical effects of treatment. At 100 mg/kg bw, five males and six females died during the study and there were some clinical signs of cholinesterase inhibition (described as fibrillations). The deaths occurred between two and seven doses. All the rats at 25 mg/kg bw gained weight, as did most of the surviving rats at 100 mg/kg bw.

No changes in clinical pathology were seen in rats at 25 mg/kg bw. At 100 mg/kg bw, males showed an increase in protein levels in pooled urine after the 14th and 27th doses, while there were no changes in the females. Examination of blood and bone marrow did not show any evidence of toxicity at either dose. Histological examination of the group receiving a dose at 100 mg/kg bw revealed no changes, so the group at 25 mg/kg bw was not examined.

In the segment of the study in which cholinesterase activity was analysed, no significant inhibition of erythrocyte, plasma or brain cholinesterase activity was detected in animals at 1.5 or 5 mg/kg bw. After 15 doses, there was no evidence of any inhibition of erythrocyte or plasma cholinesterase in rats at 25 mg/kg bw, but after 28 doses there was significant inhibition (55%) of plasma cholinesterase in female rats. At 100 mg/kg bw, there was significant inhibition of plasma cholinesterase in females after both 14 doses (41%) and 28 doses (63%). Brain and erythrocyte cholinesterase activity in the females and erythrocyte, plasma and brain cholinesterase activity in the males were not inhibited after either 14 or 28 doses. The NOAEL was 25 mg/kg bw on the basis of increased urinary protein at 100 mg/kg bw per day (Parkinson, 1979).

The plant carbamate metabolite desmethylformamidopirimicarb (purity, >98% w/w; R34885; 062/04, batch No. R34885P) was studied for its general toxic and haematological effects and its inhibitory effects on cholinesterase activity in Alderley Park SPF albino rats given repeated doses. The study consisted of two parts. For the toxicity and haematological investigation, groups of 10 male and 10 female rats were given desmethylformamidopirimicarb (R348385) at a dose of 12.5 or 50 mg kg bw per day by gavage. A control group of five males and five females was given the vehicle only ("Lissatan" AC). Half the animals at each dose were given 14 consecutive daily doses and the remainder were given 28 consecutive daily doses. General observations, body weight, blood and urine

biochemistry and haematological parameters were measured. At the end of the study, all the rats were examined post mortem. Selected tissues were examined histopathologically.

For the assessment of cholinesterase inhibition, five males and five females of each sex per group were given R34885 at a dose of 0, 3 or 12.5 mg/kg bw per day for 28 days. Blood samples were taken from all rats before dosing, 1 h after the 14th dose and 1 h after the 28th dose for measurement of erythrocyte and plasma cholinesterase activity. Brain cholinesterase activity was measured at autopsy.

In the first part of the study, no clinical abnormalities were seen at either dose and all rats gained weight during the course of the study. No compound-related changes were seen in the blood or urine of any animals at either dose. Examination of blood and bone marrow did not show any evidence of toxicity at either dose. Histological examination of the group of animals at 50 mg/kg bw revealed no changes, so the group at 12.5 mg/kg bw was not examined.

In the part of the study in which cholinesterase activity was investigated, no significant inhibition of erythrocyte, plasma or brain cholinesterase activity was detected in animals at 3 mg/kg bw. At 12.5 mg/kg bw there was no evidence of any significant effect on erythrocyte or brain cholinesterase activity after 14 or 28 doses, or of any effect on plasma cholinesterase after 14 doses. There was significant inhibition (46%) of plasma cholinesterase activity in females after 28 doses, while there was no effect in males. The NOAEL was 12.5 mg/kg, the highest dose tested (Parkinson, 1978).

A group of 10 male and 10 female Alderley Park SPF albino rats was given the pirimicarb plant carbamate metabolite desmethyl pirimicarb (R34836; 836/01, batch No. and purity not given) at a dose of 100 mg/kg bw per day by gavage in propylene glycol on 5 days per week for 2 weeks. The rats were observed for signs of toxicity, and 24 h after the final dose, blood was taken for haematological analyses and for measurement of cholinesterase activity. All rats were examined post mortem and selected tissues from four males and four females were examined histopathologically.

Slight fibrillations, incontinence and salivation were apparent within 30 min of each dose, but these signs abated within 2 h and the rats appeared to have completely recovered within 24 h. Food consumption and body weight was unaffected, with the exception of one female that died because of a dosing accident.

There was no inhibition of brain or erythrocyte cholinesterase activity, although plasma cholinesterase, a less reliable indicator of anticholinesterase activity, was inhibited by approximately 25% in females.

Haematological examination (in comparison with "control" values rather than a usual concurrent control) showed that both males and females were slightly hypochromic (mean cell haemoglobin: males, 32.7% versus 30.6%; females, 33.3% versus 30.8%), and males had reticulocytosis (reticulocytes as a percentage of erythrocytes: 4.50 versus 7.20).

No gross abnormalities were detected at examination post mortem. At histopathological examination, four males and one female showed increased haemopoietic activity in the spleen, and haemopoietic cells were also seen in the thymus in one male and one female,

and a slightly reactive thymus was seen in one male and one female. An NOAEL could not be determined from this study, in which a single dose was tested (Fletcher, 1971a).

A group of 10 male and 10 female Alderley Park SPF albino rats were given the pirimicarb plant carbamate metabolite desmethylformamidopirimicarb (R34885, 062/04), batch No. and purity not given) at a dose of 25 mg/kg bw per day by gavage in propylene glycol on 5 days per week for 2 weeks. The rats were observed for signs of toxicity and, 24 h after the final dose, blood was taken for haematological analyses and for measurement of cholinesterase activity. All rats were examined post mortem and selected tissues from four males and four females were examined histopathologically.

No signs of toxicity were noted and there was no effect on food consumption or body weight. There was no inhibition of plasma, erythrocyte or brain cholinesterase activities 24 h after the final dose. However, it should be noted that as R34885 is a carbamate, peak inhibition would be expected 1–4 h after dosing, hence, a potential effect would have been missed in this study. This potential problem was addressed in a subsequent study (see Parkinson, 1978).

Haematological examination revealed slight hypochromia in both males and females (mean cell haemoglobin: males, 32.7% versus 31.2%; females, 33.3% versus 30.2%). However, comparison was with "normal" values rather than a concurrent control. There was no effect on clotting function. No gross abnormalities were seen at examination post mortem and the only significant histopathological abnormality seen was increased haemopoietic activity in the spleen in four males and one female. A NOAEL could not be determined from this study (Fletcher, 1971b).

The Fletcher (1971b) study was repeated with special reference to the haematological evaluation. Desmethylformamidopirimicarb (purity, >98%; R34885, 062/04, batch No. R34885P_5) was prepared as an aqueous suspension in 0.5% "Lissatan" AC and administered at a dose of 12.5 or 50 mg/kg bw per day by gavage to four groups of 10 male and 10 female Alderley Park SPF albino rats for either 14 or 28 days. Control groups o five males and five females were given the vehicle only for either 14 or 28 days. These rats were observed for signs of toxicity and 24 h after the final dose, blood was taken for haematological analyses. All rats were examined post mortem and selected tissues from animals in the group dosed at 50 mg/kg bw per day were examined histopathologically. No cholinesterase activities were measured in these rats. Parallel groups of five male and five female Alderley Park SPF albino rats were given R34885 at a dose of 0, 3 or 12.5 mg/kg bw per day for 28 consecutive days. These rats were bled (tail vein) twice in the 7 days before dosing began and then 1 h after each of the 14th and 28th doses for measurement of erythrocyte and plasma cholinesterase activity. Brain cholinesterase activity was also measured post mortem.

There were no effects of treatment on the clinical condition or behaviour of the rats, on body-weight gain, chemical analysis of blood, haematology or histopathology at either 12.5 or 50 mg/kg bw per day.

No significant inhibition of erythrocyte, plasma or brain cholinesterase activity was detected at 3 mg/kg bw per day. At 12.5 mg/kg bw per day, there was no evidence of any significant effect on the cholinesterase activity in erythrocytes after 14 or 28 doses, in plasma after 14 doses or in brain after 28 doses. There was, however, a significant, 46% inhibition

of plasma cholinesterase activity in female rats after 28 days, but there was no significant inhibition in male rats. The NOAEL for pirimicarb metabolite R34885 was 3 mg/kg bw per day on the basis of inhibition of plasma cholinesterase activity 1 h after dosing (Parkinson, 1978).

Pirimicarb metabolite R31805 has been tested in short-term studies of toxicity in vivo. As this metabolite is converted to R34865 in vivo, these studies on R31805 are also effectively testing R34865. Both metabolites have been tested individually, however, for acute oral toxicity and for genotoxicity.

Groups of 12 male and 12 female Alpk:AP$_f$SD (Wistar derived) rats were given diets containing technical-grade pirimicarb hydroxypyrimidine metabolite 2-dimethylamino-5,6-dimethylpyrimidino-4-ol (purity, 100% w/w; R31805; 062/06, batch No. P4) at a concentration of 0, 80, 240 or 800 ppm for 90 consecutive days. These dietary concentrations were equal to received doses of 0, 6.6, 19.5 or 65.6 mg/kg bw for males and 0, 7.3, 22.0 and 73.9 mg/kg bw for females. Satellite groups of four males and four females were dosed at the same dietary concentrations for 90 days and used for measurement of cholinesterase activity.

Clinical observations, body weights and food consumption were measured for all rats throughout the study. Detailed clinical observations, including quantitative assessments of landing foot splay, sensory perception and muscle weakness were made and, for all main-phase rats, assessment of motor activity was performed during week 12, urine sampling during week 13 and ophthalmoscopy during weeks −1 and 13. At the scheduled end of the exposure period, all rats were killed and the main phase animals were subjected to a full examination post mortem. Cardiac blood samples were taken for clinical pathology, selected organs were weighed and specified tissues were taken for subsequent histopathological examination.

There were no treatment-related effects on body weight or food consumption and there were no treatment-related clinical signs, ophthalmoscopic findings or effects on the FOB.

Among males at 800 ppm, mean motor activity was higher from 36–50 min and overall compared with values for concurrent controls. However, in the absence of any associated clinical signs or pathology findings, the increase in motor activity was considered to be of no toxicological significance.

Plasma cholesterol concentrations were increased in males receiving 2-dimethylamino-5,6-dimethylpyrimidino-4-ol (R31805) at a dietary concentration of 800 ppm and in females at all doses. Plasma total protein was increased in males at 800 ppm and triglycerides were increased in both sexes at this dose. Increases were seen in alanine aminotransferase and aspartate aminotransferase activities in females at 240 and 800 ppm. In the absence of any associated adverse pathology findings, these changes in blood chemistry parameters were considered to be of no toxicological significance.

There were no treatment-related effects on haematology or urine clinical chemistry parameters and no evidence of inhibition of cholinesterase activity.

There were no treatment-related pathology findings. After adjustment for final body weight, group mean liver weights were statistically significantly higher than those of

controls for males receiving 2-dimethylamino-5,6-dimethylpyrimidino-4-ol (R31805) at a dietary concentration of 800 ppm and for females at 240 ppm and 800 ppm. However, in the absence of any associated pathology findings in the liver, the increased liver weights may indicate a slight adaptive change and are considered unlikely to be of toxicological importance.

The NOAEL for 2-dimethylamino-5,6-dimethylpyrimidino-4-ol (R31805) was 240 ppm, equal to 19.5 mg/kg bw per day, on the basis of blood chemistry changes at 800 ppm, equal to 65.6 mg/kg bw per day (Lees, 2001).

Genotoxicity of metabolites

2-dimethylamino-5,6-dimethylpyrimidino-4-ol (R31805; 062/06)

The pirimicarb metabolite 2-dimethylamino-5,6-dimethylpyrimidino-4-ol (R31805; 062/06) was tested for genotoxicity in a number of assays, both in vitro and in vivo (Table 34). In *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E.coli* strains WP2P and WP2P *uvr*A no significant response was observed (Callander, 2000a), while it did induce significant responses in L5178Y $Tk^{+/-}$ cells, both in the presence of metabolic activation (S9) with treatment for 4 h and in the absence of S9 with treatment for 24 h (Clay, 2001a). No significant response was observed in a test for clastogenicity using cultured human lymphocytes treated in vitro in either the presence or absence of metabolic activation (Fox, 2001a).

No significant responses were observed in an assay for unscheduled DNA synthesis assay in vivo/in vitro in rat liver cells (Clay, 2001c) or an assay for micronucleus formation in bone marrow in mice (Clay, 2001d).

5,6-dimethyl-2-(methylamino)pyimidin-4-ol (R34865; 062/07)

The pirimicarb metabolite 5,6-dimethyl-2-(methylamino)pyimidin-4-ol (R34865; 062/07) was tested for genotoxicity in a number of assays, both in vitro and in vivo (Table 35). In *S. typhimurium* strains TA1535, TA1537, TA98 and TA100 and *E.coli* strains WP2P

Table 35. Results of studies of genotoxicity with pirimicarb metabolite 2-dimethylamino-5,6-dimethylpyrimidino-4-ol (R31805; 062/06)

End-point	Test object	Dose (LED/HID)	Reference No./Purity (%)	Result	Reference
Gene mutation in vitro	*S. typhimurium* strains TA100, TA1535, TA1537, TA98; *E.coli* WP2, WP2 *uvrA* (±S9 mix)	5000 µg/plate	P4/100	Negative	Callander (2000a)
Gene mutation in vitro	Mouse lymphoma L5178Y cells, Tk^+ locus (±S9)	125 µg/ml –S9 (24 h) 500 µg/ml +S9 (4 h)	P4/100	Positive (24 h) Positive (4 h)	Clay (2001a)
Chromosomal aberration in vitro	Human lymphocytes (±S9)	1670 µg/ml	P4/100	Negative	Fox (2001a)
Unscheduled DNA synthesis in vivo/in vitro	Male Alpk:APfSD rat liver cells 4 & 12h after dosing	200 mg/kg bw per os × 1	P4/100	Negative	Clay (2001c)
Micronucleus formation in vivo	Male C57BL/6JfCD-1/Alpk mouse bone-marrow cells 24, 48 & 72 h after dosing	125 mg/kg bw, per os × 1	P4/100	Negative	Clay (2001d)

LED, lowest effective dose; HID, highest ineffective dose

Table 36. Results of studies of genotoxicity with pirimicarb metabolite 5,6-dimethyl-2-(methylamino)pyimidin-4-ol (R34865; 062/07)

End-point	Test object	Dose (LED/HID)	Reference No./purity (%)	Result	Reference
Gene mutation in vitro	*S. typhimurium* strains TA100, TA1535, TA1537, TA98; *E.coli* WP2, WP2 *uvrA* (±S9)	5000 µg/plate	P5/100	Negative	Callander (2000b)
Gene mutation in vitro	Mouse lymphoma L5178Y cells, Tk^+ locus (±S9)	500 µg/ml −S9 (24 h)	P5/100	Positive (24 h)	Clay (2001b)
		1530 µg/ml +S9 (4 h)		Negative	
Chromosomal aberration in vitro	Human lymphocytes (±S9)	500 µg/ml	P5/100	Negative	Fox (2001b)

LED, lowest effective dose; HID, highest ineffective dose

and WP2P *uvr*A, no significant response was observed (Callander, 2000b), while it did induce significant responses in L5178Y $Tk^{+/-}$ cell assay in the absence of metabolic activation with treatment for 24 h; no significant responses were observed in this assay in either the presence or absence of metabolic activation after treatment for 4 h (Clay, 2001b). No significant response was observed in a test for clastogenicity using cultured human lymphocytes treated in vitro in either the presence or absence of metabolic activation (Fox, 2001b).

3. Observations in humans

The symptoms of carbamate poisoning are similar to those of organophosphorus poisoning, i.e. they occur as a result of inhibition of acetyl cholinesterase activity, although they are less severe and with a much more rapid onset and shorter duration of effect. Typical features are excessive salivation and pupillary constriction in less severe cases, with muscular twitching, incontinence, convulsions and death from respiratory muscle paralysis in more severe cases (Echobichon, 2001).

In cases of inhalational or dermal overexposure, these incapacitating symptoms tend to reduce further exposure by preventing the operator from working. Recovery is rapid and complete, and there is no evidence to suggest chronic or cumulative effects. In cases of suspected poisoning, a blood sample should be taken for analysis of whole blood or erythrocyte cholinesterase activity. Up to 40% cholinesterase inhibition may occur without evidence of systemic symptoms (Lotti, 2000).

The Stewardship Department of Syngenta (formerly Zeneca/ICI Agrochemicals), which includes the occupational health function, has maintained a database of incidents involving chemical exposure of workers since 1983. At the time it was set up it was used to formally record reports of clinical conditions arising during work at the company's research station (at Jealott's Hill, Berkshire) and formulation plant (at Yalding, Kent). Information is gathered at the time of the incident and is recorded on a standard form by the occupational health nurse or the doctor. From the start of 1994, such data have been collected from all sites around the world, using a pro-forma.

Interrogation of the database revealed one report of adverse reactions from a product containing pirimicarb. This was a case of neck skin irritation in a filling and packing operator.

In a report on the medical surveillance of workers involved in production of pirimicarb formulations from 1969 to 1973 at the ICI Yalding factory, Maidstone, United Kingdom (Bagness et al., 1975), blood cholinesterase activities were routinely investigated each week for plasma and erythrocyte activities. Baseline values were established on two to four samples taken before exposure. Inhibitions of 20% were considered within normal limits, with inhibition of > 30% resulting in movement of workers.

During 1969, two of five workers exposed to pirimicarb (for 9–144 h) showed inhibition of cholinesterase activity of >30%. These two workers had processed 14 or 32 tons of Pirimor (an industrial formulation of pirimicarb), and the second, who had been exposed for approximately 144 h, reported headache and nausea. Both cases were attributed to plant failure leading to exposure to pirimicarb dust. Half suits (details not provided) were reportedly worn during charging and filling operations. A third worker was moved after having processed 16 tons of Pirimor because he was feeling unwell, but without showing cholinesterase inhibition.

During 1970, full air suits were worn during such work, with 10 workers exposed to pirimicarb for 8–25 h. No problems with illness or cholinesterase depression were encountered.

During 1971–1973, a new formulation plant designed to minimize production of airborne dust and using exhaust ventilation was used for the production of Pirimor. Depressions of, principally plasma, cholinesterase activity of >20% were detected on numerous occasions.

Workers were often moved when these depressions exceeded 30%. No cases of illness were reported and cholinesterase activity returned to normal limits within days.

In 1972–73, 150 tons of Pirimor were produced during 120, 8 h shifts. On one occasion a worker was removed with depression of plasma and erythrocyte cholinesterase activity caused by a failure of containment equipment.

During the 1973 production of the 50% WG formulation, two workers employed continuously with pirimicarb were monitored. Inhibition of cholinesterase activity (generally <20% reduction in erythrocytes and up to 69% reduction in plasma) was not prevented by the engineering, personal protection equipment (gloves, overalls, ori-nasal dust masks or filtered air hood) and hygiene systems. This failure was attributable to exposure to pirimicarb vapour from the granulation and fluidized bed drying process. When these were ventilated and personal protective hoods (supplied with fresh air) were used, no depression of cholinesterase activity or signs of illness were seen.

Comments

Kinetic studies in rats have demonstrated that pirimicarb administered orally to male and female rats is rapidly and extensively absorbed (>70% of the administered dose) and widely distributed. Radioactivity from [^{14}C]pyrimidinyl-labelled pirimicarb was excreted predominantly in the urine, while radioactivity from [^{14}C]carbamoyl-labelled pirimicarb was excreted predominantly in expired air. Tissue retention of radioactivity was low. There were no pronounced sex differences in the routes or rates of excretion. Pirimicarb was extensively metabolized, giving rise to 24 metabolites, 17 of which were identified. The

major metabolic pathway involves the loss of the carbamate moiety to produce a range of substituted hydroxypyrimidines, some of which are glucuronide conjugates.

The acute oral LD_{50} for pirimicarb was 152 mg/kg bw in male rats and 142 mg/kg bw in female rats, while the acute dermal LD_{50} of pirimicarb was >2000 mg/kg in both male and female rats. The 4 h inhalation LC_{50} of pirimicarb in rats was 0.948 mg/l of air in males and 0.858 mg/l of air in males and females respectively. Pirimicarb is not irritating to the rabbit eye or skin. It does, however, have skin sensitizing potential under the conditions of the Magnusson & Kligman maximization test.

In a 21-day study of dermal toxicity in rats, there were no signs of irritation and no clinical signs of systemic toxicity, but there was a reduction in brain cholinesterase activity at 1000 mg/kg bw per day. The NOAEL was 200 mg/kg bw per day.

Acetylcholinesterase that has been inhibited by pirimicarb is rapidly reactivated (both in vivo and in vitro). This property hampers the reliable determination of acetylcholinesterase inhibition in erythrocytes and brain in treated animals, and special attention must be given to crucial methodological features (e.g. time between sampling and measurement, sample temperature and dilution). Consequently, the most reliable indicators of an effect are clinical signs, which usually occur when acetylcholinesterase inhibition is >50% at nerve terminals.

In experiments with multiple doses, common toxicological targets are blood and acetylcholinesterase activity.

Three dietary studies of up to 90 days in duration have been conducted in rats. In the first study (in which animals were given diets containing pirimicarb at a concentration of 250 or 750 ppm for 90 days) there were no adverse clinical, haematological or other pathological effects. A reduction in plasma cholinesterase activity was seen at 750 ppm, providing evidence for the absorption of the compound from the intestinal tract. In the second study (in which animals were given diets containing pirimicarb at a concentration of 250 or 750 ppm for 8 weeks) a clear reduction in body-weight gain was seen at 750 ppm, with a slight reduction at 250 ppm. These growth reductions were completely reversible after an 8-week recovery period. In the final study (in which animals were given diets containing pirimicarb at a concentration of 100, 175, 250 or 750 ppm for 8 weeks) there were no adverse clinical effects. A reduction in body-weight gain and food consumption was seen at 750 ppm. Owing to the effect on body-weight gain in the second study at 250 ppm, the Meeting concluded that the overall NOAEL in short-term studies in rats was 175 ppm, equivalent to 17.5 mg/kg bw per day.

Reports of three studies of 13–16 weeks in duration in dogs were available. In the first study, beagle dogs were given diets delivering pirimicarb at a dose of 0, 4, 10 or 25 mg/kg bw per day for at least 90 days. Body weight was reduced at the highest dose and plasma cholinesterase activity was reduced at 10 and 25 mg/kg bw per day. Bone-marrow changes, indicative of increased erythropoiesis, were observed in the terminal blood films in all treatment groups. Three out of 32 animals (two receiving 25 mg/kg bw per day and one receiving 10 mg/kg bw per day) developed anaemia. Two dogs of each sex per group were killed after 90 days (one male only in the group receiving 25 mg/kg bw per day, as one had been killed after 10–11 weeks) and the remainder were allowed to recover untreated for 28 days. Partial recovery from the bone-marrow changes was evident at 28 days after cessation of

treatment. A NOAEL for this study was not identified, therefore a second study with diets delivering pirimicarb at a dose of 0.4, 1.8 and 4 mg/kg bw per day for at least 90 days was conducted. An additional group received pirimicarb at 4 mg/kg bw per day for 180 days. There were no adverse clinical or pathological effects, but dogs at 4 mg/kg bw per day showed evidence of increased erythropoietic activity in the bone marrow. The NOAEL was 1.8 mg/kg bw per day. In the third study, foxhounds were given diets delivering pirimicarb at a dose of 0, 2 or 25/50 mg/kg bw per day for 16 weeks, followed by a 7-week recovery period. The dose of 25 mg/kg bw per day was increased to 50 mg/kg bw per day from week 8. Anaemia and reticulocytosis developed in dogs receiving a dose of 50 mg/kg bw per day, and bone-marrow changes, characterized by an increase in normoblasts and hypoplasia, were observed. Both the anaemia and bone-marrow changes were reversible when the dose was reduced to 25 mg/kg bw per day, or upon cessation of treatment. The NOAEL in this study in dogs was 2 mg/kg bw per day.

In addition, there were two 2-year dietary studies in dogs, and a more recent, guideline-compliant, 1-year study in which pirimicarb was administered in capsules. In the first 2-year dietary study, designed to reproduce and characterize the anaemia, two out of four unrelated beagles had an immune haemolytic anaemia when exposed to pirmicarb at a dose of 25 or 50 mg/kg bw per day for at least 3 months. Dogs at up to and including 2 mg/kg bw per day did not show such effects. The anaemia was completely reversible after withdrawal of the compound. Other dogs in the same study showed no haematological changes when exposed to primicarb at a dose of up to 50 mg/kg bw per day for 2 years. In the second dietary study, pirimicarb was administered at a dose of 0.4, 1.8 or 4 mg/kg bw per day for 2 years. There were no adverse changes in growth rate, blood and urine clinical chemistry, organ weights or histopathology. At 4 mg/kg bw per day, there were reductions in haemoglobin concentration and erythrocyte volume fraction in males, and a slight increase in the erythroid to myeloid ratio in two females. No adverse changes were detected in the bone marrow. None of the dogs developed overt anaemia. In the most recent study, groups of beagles were dosed orally with gelatine capsules containing pirimicarb at a dose of 0, 3.5, 10 or 25/35 mg/kg bw per day for 1 year. The highest dose of 35 mg/kg bw per day could not be sustained owing to adverse clinical signs in week 1, so from week 4 onwards, the dose was reduced to 25 mg/kg bw per day. One female dog receiving 25 mg/kg bw per day was killed humanely in week 36 after significant body-weight loss and the development of anaemia. The haematological changes in this dog were characterized by increased erythropoietic activity in the bone marrow and by histological changes consistent with increased erythrocyte breakdown. No other dog showed any treatment-related haematological changes; however, increased haemosiderin deposition was observed in the liver and spleen of dogs at 25 mg/kg bw per day. The NOAEL in this study was 3.5 mg/kg bw per day; this value is very close to the LOAEL of 4 mg/kg bw identified in three other experiments. The overall NOAEL was 2 mg/kg bw per day in dogs.

The carcinogenic potential of pirimicarb has been assessed in feeding studies of 80 and 96 weeks duration in mice and 104 weeks in rats. In both species, the highest dose tested induced moderate levels of toxicity. Two studies of carcinogenicity were conducted in Alderley Park Swiss-derived mice and one with in C57 black Alderley Park mice; however, one of the studies in Swiss-derived mice, a study that pre-dated the establishment of GLP, was not considered adequate for the assessment of the carcinogenic potential of pirimicarb, owing to a high incidence of respiratory disease. Similarly, three pre-GLP studies in rats were not adequate for carcinogenicity assessment owing to high incidences of respiratory disease.

In Alderley Park Swiss-derived mice given diets containing pirimicarb at a concentration of 0, 0, 200, 400 or 1600 ppm for up to 96 weeks, there was a significant increase in the incidence of liver tumours classified as type A (hyperplastic nodules and benign neoplasms) and type B nodules (which showed characteristics of malignancy) at the highest dose (equivalent to approximately 240 mg/kg bw per day), with no evidence of nodule induction at lower doses. The incidence of liver nodules was above the range for historical controls for the test laboratory. This finding was not confirmed in C57 black Alderley Park mice given diets containing pirimicarb at a concentration of 0, 50, 200 or 700 ppm for at least 80 weeks, where there was no evidence that liver tumours induced by pirimicarb at doses of up to 700 ppm (equal to approximately 94 and 130 mg/kg bw per day in males and females, respectively). Thus, the significant response was only seen at a very high dose and after a prolonged exposure time in a mouse strain with a high and variable background incidence of liver tumours.

Also in Alderley Park Swiss-derived mice, the incidence of pulmonary adenoma was significantly increased in both sexes at the highest dose, but with no significant response at lower doses. Data from concurrent and historical controls indicated a high and variable spontaneous background incidence of pulmonary adenomas in this strain of mouse. Given the overall variability in the incidence of pulmonary adenoma in these mice, the observation of an increased tumour incidence at the highest dose does not give cause for concern in terms of cancer risk. A small, statistically significant, increase in the incidence of pulmonary adenoma was also observed in female (but not male) C57 black Alderley Park mice at the highest dose tested, with no evidence of pulmonary adenoma induction at lower doses. In contrast to Swiss-derived mice, C57 black mice have a low spontaneous background incidence of pulmonary adenoma. Therefore, the occurrence of these tumours is considered to be treatment-related. The absence of a significant response in male mice could be a chance difference in the incidence of an uncommon tumour type. The Meeting concluded that oral administration of pirimicarb at a dose of up to 700 ppm, equal to 94 and 130 mg/kg bw per day for males and females, respectively, for at least 80 weeks produced a small increase in the incidence of benign lung tumours in females, but not in males. The NOAEL for non-neoplastic effects was 50 ppm, equal to 6.7 mg/kg bw per day, on the basis of slight haematological changes at 200 ppm, equal to 26.6 mg/kg bw per day in the 80-week study.

A 2-year study in rats showed that dietary administration of pirimicarb at 0, 75, 250 or 750 ppm resulted in reduced body-weight gains and food consumption in both sexes at 750 ppm, indicating that a maximum tolerated dose had been achieved. There was also a slight reduction in body-weight gain at 250 ppm in females. There were increases in plasma cholesterol at all observation times at 750 ppm, at weeks 13 and 26 at 250 ppm and (in females only) at week 13 at 75 ppm. Plasma concentrations of triglycerides were increased at 750 ppm in males at weeks 52 and 78, and in females at weeks 13 and 26. Males fed diets containing pirimicarb at 750 ppm showed a small increase in incidence and severity of necrosis in the brain. The significance of this is equivocal, but could not be dismissed as being incidental to treatment with pirimicarb. Females fed diets containing pirimicarb at 750 ppm showed an increased severity of sciatic nerve demyelination and an increased severity and incidence of voluntary muscle degeneration that were considered to be an exacerbation of a spontaneous age-related change. Overall, the findings in the brain, sciatic nerve and voluntary muscle were minor, confined to the highest dose and did not elicit any clinical signs of increased neurological dysfunction. Plasma cholinesterase activity was slightly reduced in females at 250 and 750 ppm, demonstrating the absorption of the test substance, but brain and erythrocyte cholinesterase activities were not affected at any dose. The

NOAEL for non-neoplastic effects was 75 ppm, equal to 3.7 mg/kg bw per day, on the basis of reductions in body weights and increases in plasma cholesterol and triglycerides at 250 ppm, equal to 12.3 mg/kg bw per day. There was an overall higher, but non-significant incidence in the number of male rats with tumours at 250 and 750 ppm. This reflected an increased incidence of males with multiple tumours at 250 ppm and of males with single tumours at 750 ppm. There were also small increased incidences of astrocytoma of the brain in all treated groups and in females at 750 ppm, but these were not statistically significant and no dose–response relationship was evident. As there was a decreased incidence of males with multiple tumours at 750 ppm and there were no consistent effects across doses, the Meeting concluded that pirimicarb did not induce a carcinogenic response in any tissue.

The Meeting concluded that pirimicarb did not demonstrate clear evidence of carcinogenic potential in mice or rats. The liver tumours were not consistently found in the two studies in mice, while the benign lung tumours were found only at the highest dose and with clear evidence for a threshold. There were no compound-related increases in any tumour type in rats.

Pirimicarb was tested for genotoxicity in an adequate range of studies, both in vitro and in vivo. The results observed were largely negative. A small increase in mutant frequency in the assay for mutation in L5178Y mammalian cells, in the presence of metabolic activation, was considered not to be a significant alert for genotoxicity. Pirimicarb has shown no evidence of genotoxic potential in several test systems in vivo.

The Meeting concluded that pirimicarb is unlikely to pose a genotoxic risk to humans.

Because the results of the studies of carcinogenicity in rodents were judged not to provide evidence of carcinogenic potential, an evaluation supported by the lack of genotoxic potential, the Meeting concluded that pirimicarb is unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, the NOAEL for adult rats and for their offspring was 200 ppm, equal to 23 mg/kg bw per day in adults, on the basis of systemic toxicity in the parental rats and reduced body-weight gain in the parental rats and the offspring at a dose of 750 ppm, equal to 88 mg/kg bw per day; no other signs of reproductive toxicity were observed at this dose, the highest tested. In studies of developmental toxicity in rats, the NOAEL for fetal toxicity and maternal toxicity was 25 mg/kg bw per day on the basis of reduced fetal weight and maternal body-weight gains at 75 mg/kg bw per day. In studies of developmental toxicity in rabbits, the NOAEL for fetal and developmental toxicity was 60 mg/kg bw per day, the highest dose tested, and the NOAEL for maternal toxicity for 10 mg/kg bw per day, on the basis of reduced food consumption and body-weight gains at 60 mg/kg bw per day. The results from the two studies of developmental toxicity and the study of reproductive toxicity demonstrated that fetuses and pups were not more susceptible than adults to toxicity caused by pirimicarb.

In a study of acute neurotoxicity in rats, a single oral administration of pirimicarb at 110 mg/kg bw per day by gavage resulted in early mortalities, adverse clinical signs and reductions in brain, erythrocyte and plasma cholinesterase activities. These clinical and enzyme activity changes were transient and were not associated with histopathological changes in the nervous system. At a dose of 40 mg/kg bw, there was evidence of toxicity seen as a single mortality, transient adverse clinical signs in a few rats and reduced

motor activity on day 1. Plasma cholinesterase activity was reduced, but this observation was not accompanied by biologically significant reductions in brain or erythrocyte cholinesterase activity at this dose. The Meeting concluded that the NOAEL for acute neurotoxic potential was 10 mg/kg bw per day and this value formed the basis for the ARfD. The acute toxic effects of pirimicarb are due to inhibition of acetylcholinesterase activity at nerve terminals. Inhibition of acetylcholinesterase by carbamates (such as pirimicarb) and organophosphates involves the carbamoylation or phosphorylation of the active site on the enzyme. Plasma cholinesterase is inhibited by a similar mechanism; therefore, although this is a toxicologically irrelevant target, its inhibition serves as an indicator of exposure and a surrogate for the response of acetylcholinesterase. The degree of enzyme inhibition is dependent on the concentration of inhibitor, a property that is particularly significant for carbamates because of the short occupation half-life at the active site of the enzyme (a few minutes, both in vitro and in vivo). Consequently, plasma cholinesterase activity was inhibited after a single dose of pirimicarb at 25 mg/kg bw by gavage, but not after dietary exposure corresponding to a daily dose of about 40 mg/kg bw, when the C_{max} would have been lower.

In a 90-day study of neurotoxicity, rats fed diets containing pirimicarb at a concentration of 250 or 1000 ppm resulted in toxicity evident as reduced growth and food consumption or /utilization. There were no treatment-related effects on the functional observational battery, motor activity, cholinesterase and neurotoxic esterase activities or neuropathology. The NOAEL for neurotoxicity in this study was 1000 ppm, equal to 77 mg/kg bw per day, the highest dose tested.

Studies of toxicity have been conducted on a number of metabolites of pirimicarb: three carbamate metabolites, three hydroxypyrimidine metabolites, and three guanidine metabolites. The acute toxicities of two carbamates (the desmethyl pirimicarb and the desmethylformamido pirimicarb metabolites) were of the same order as that of pirimicarb itself, while the LD_{50}s of all the other seven metabolites were less or considerably less than that of pirimicarb itself. In addition, some of these metabolites have been subjected totested in studies of toxicity after with repeated doses and some to assays for genotoxicity. Desmethyl pirimicarb and desmethylformamido pirimicarb had effects on cholinesterase that were similar to those caused by pirimicarb itself and are included in the residue definition, since they occur in plants. In 28- and 90-day studies of toxicity in rats, the hydroxypirimidine metabolite, 2-dimethylamino-5,6-dimethylpyrimidin-4-ol (and, by implication, its mammalian metabolite, 5,6-dimethyl-2-(methylamino)pyrimidin-4-ol) was of low toxicity, the NOAEL being was 240 ppm, equal to 19.5 mg/kg bw per day, on the basis of blood chemistry changes at 800 ppm, equal to 65.6 mg/kg bw per day. NOAELs could not be identified because testing was restricted to single doses in the cases of desmethyl pirimicarb (100 mg/kg bw per day for 2 weeks) and desmethylformamido pirimicarb (25 mg/kg bw per day for 2 weeks). Each of these metabolites caused slight hypochromia (reduced haemoglobin concentrations per cell). Genotoxicity tests were conducted with the hydroxypirimidines, 2-dimethylamino-5,6-dimethylpyrimidin-4-ol and 5,6-dimethyl-2-(methylamino)pyrimidin-4-ol. Both metabolites, like pirimicarb itself, produced some weak evidence of mutagenic effects in the assay in mouse lymphoma cells, but not in other assays. The Meeting concluded that, within the limitations of studies conducted (short-term and only in rats), desmethyl pirimicarb and desmethylformamido pirimicarb have toxicological profiles similar to that of pirimicarb itself.

The Meeting concluded that the existing database on pirimicarb was adequate to characterize the potential hazards to fetuses, infants and children.

There have been reports of production workers with inhibition of plasma and erythrocyte cholinesterase activity of sufficient severity to result in their movement to other work areas.

Toxicological evaluation

An ADI of 0–0.02 mg/kg bw was established for pirimicarb and its dimethyl carbamate metabolites on the basis of the overall NOAEL of 2 mg/kg bw per day in 90-day and 2-year studies in dogs treated by dietary administration, and with a safety factor of 100.

The Meeting established an ARfD of 0.1 mg/kg bw for pirimicarb on the basis of a NOAEL of 10 mg/kg bw in a study of acute neurotoxicity in rats. Although a reduced safety factor would be supported by the reversibility of clinical signs and the C_{max}-dependency of the effects, a safety factor of 100 was used in consideration of the steep dose–response curve (indicated by a a mortality at the LOAEL) and the lack of reliable measurement of acetylcholinesterase inhibition. Haematotoxicity in dogs was also considered as a possible end-point for an ARfD; however, in one study in dogs haematological parameters were measured during treatment before the onset of anaemia, indicating that this condition did not occur after a single dose.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-month and 21-month studies of toxicity and carcinogenicity[a]	Toxicity	50 ppm, equal to 6.7 mg/kg bw per day	200 ppm, equal to 27 mg/kg bw per day
		Carcinogenicity	200 ppm, equal to 37mg/kg bw per day	700 ppm, equal to 94 mg/kg bw per day
Rat	24-month study of toxicity and carcinogenicity[a]	Toxicity	75 ppm, equal to 3.7 mg/kg bw per day	250 ppm, equal to 12.3 mg/kg bw per day
		Carcinogenicity	750 ppm, equal to 37 mg/kg bw per day[c]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	200 ppm, equal to 22 mg/kg bw per day	750 ppm, equal to 88 mg/kg bw per day
		Offspring toxicity	200 ppm, equal to 23 mg/kg bw per day	750 ppm, equal to 88 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	25 mg/kg bw per day	75 mg/kg bw per day
		Embryo- and fetoal toxicity	25mg/kg bw per day	75 mg/kg bw per day
	Single-dose neurotoxicity[b]	Neurotoxicity	10 mg/kg bw	40 mg/kg bw per day
	3-month study of neurotoxicity[a]	Neurotoxicity	1000 ppm, equal to 81 mg/kg bw per day[c]	—
Rabbit	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	60 mg/kg bw per day
		Embryo and fetal toxicity	60 mg/kg bw per day[c]	—
Dog	90-day and 2-year studies of toxicity[a]	Toxicity	2 mg/kg bw per day	4 mg/kg bw per day

[a] Dietary administration
[b] Gavage administration
[c] Highest dose tested

Estimate of acceptable daily intake for humans

0–0.02 mg/kg bw

Estimate of acute reference dose

0.1 mg/kg bw

Studies that would provide information useful to the continued evaluation of the compound

Further observations in humans

Summary of critical end-points for pirimicarb

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of oral absorption	Rapid; >80% absorbed
Dermal absorption	No study of direct dermal absorption available, but brain cholinesterase activity was inhibited after application of pirimicarb to the skin of rats, indicating absorption by this route
Distribution	Distributed throughout the body; highest concentrations in liver and fat
Potential for accumulation	Low, owing to rapid excretion
Rate and extent of excretion	Rapid, >80% excretion within 24 h
Metabolism in animals	Extensive
Toxicologically significant compounds (animals, plants and environment)	Parent and the metabolites desmethyl pirimicarb and desmethylformamido pirimicarb
Acute toxicity	
Rat, LD_{50}, oral	142 mg/kg bw
Rat, LC_{50}, inhalation	0.858 mg/l (4 h)
Rabbit, LD_{50}, dermal	>2000 mg/kg bw
Rabbit, skin irritation	Not irritiating
Rabbit, eye irritation	Not irritating
Skin sensitization	Sensitizing (Magnusson and Kligman test)
Short-term studies of toxicity	
Target/critical effect	Body-weight gain decrement, haemolytic anaemia or cholinesterase inhibition
Lowest relevant oral NOAEL	1.8 mg/kg bw per day: (3_month study in dogs)
Lowest relevant dermal NOAEL	2000 mg/kg bw per day (21_day study in rats)
Lowest relevant inhalation NOAEC	No data available
Genotoxicity	No genotoxic potential: negative in vivo, one study gave positive results in vitro
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Blood/anaemia, increased plasma lipids
Lowest relevant NOAEL	2 mg/kg bw per day (24-month study in dogs) 3.7 mg/kg bw per day (24_month study in rats)
Carcinogenicity	Benign lung tumours in mice induced by a non-genotoxic mode of action; a clear NOAEL was identified; therefore pirimicarb is unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Reproductive target/critical effect	Reduced parental and offspring body weight, clinical signs
Lowest relevant reproductive NOAEL	23 mg/kg bw per day
Developmental target/critical effect	Not teratogenic; reduced fetal body weight at maternally toxic doses
Lowest relevant developmental NOAEL	25 mg/kg bw per day (rat)
Neurotoxicity/delayed neurotoxicity	
Target/critical effect	Nervous system/cholinergic signs
Lowest relevant NOAEL	10 mg/kg bw
90-day neurotoxicity	
Target/critical effect	Nervous system/cholinergic signs
Lowest relevant NOAEL	77 mg/kg bw per day
Other toxicological studies	Desmethyl pirimicarb and desmethylformamido pirimicarb have acetylcholinesterase-inhibiting activity in rats (no studies in dogs)
Medical data	There have been a few reports of cholinesterase inhibition in workers exposed during manufacture

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.02 mg/kg bw	Dog; haemaotological changes in short- and long-term studies	100
ARfD	0.1 mg/kg bw	Rat; mortality and clinical signs of neurotoxicity in a study of acute neurotoxicity	100

References

Bagness, J.E., Harmer, C.M. & Willis, G.A. (1975) Observations on operators during formulation. ICI Plant Protection Division. Unpublished report No. TMF 1126 C (study dates, 1969–1973).

Bakemeier, R.F. & Leddy, J.P. (1968) Erythrocyte autoantibody associated with alpha-methyldopa: heterogeneity of structure and specificity. *Blood*, **32**, 1–14.

Banham, P.B., Carter, A. & James, J. (1980) Pirimicarb diet analysis: analytical investigations associated with the lifetime feeding study in the mouse (PM0002). Unpublished report No. CTL/P/511 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Before guidelines and before GLP.

Baron, R.L. (1991) Carbamate insecticides. In: Hayes, W.J. & Laws, E.R., eds, *Handbook of Pesticide Toxicology, Vol. 3. Classes of Pesticides*. New York: Academic Press, pp. 1125.

Brown, A. (1997a) Pirimicarb: whole body autoradiography in the rat. Unpublished report No. CTL/P/5383 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Zeneca Agrochemicals. Conducted according to OECD guideline 417 (1984), 87/302/EEC B.36 (1987), Agricultural Chemical Laws & Regulations, Japan (1985), US EPA Pesticide Guidelines Subdivision F, reference No. 85-1 (1994). GLP compliant.

Brown, A. (1997b) Pirimicarb: excretion and tissue distribution of a single oral dose (1 mg/kg) in the rat. Unpublished Report No. CTL/P/5329 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Zeneca Agrochemicals. Conducted according to OECD guideline 417 (1984), 87/302/EEC B.36 (1987) Agricultural Chemical Laws & Regulations, Japan (1985), US EPA Pesticide Guidelines Subdivision F, reference No. 85-1 (1994). GLP compliant.

Brown, A. (1997c) Pirimicarb: excretion and tissue distribution of a single oral dose (50 mg/kg) in the rat. Unpublished report No. CTL/P/5381 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 417 (1984), 87/302/EEC B.36 (1987), Agricultural Chemical Laws & Regulations, Japan (1985), US EPA Pesticide Guidelines Subdivision F, reference No. 85-1 (1994). GLP compliant.

Brown, A. (1997d) Pirimicarb: excretion and tissue distribution of a single oral dose (1 mg/kg) in the rat following repeat dosing. Unpublished Report No. CTL/P/5382 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 417 (1984), 87/302/EEC B.36 (1987), Agricultural Chemical Laws & Regulations, Japan (1985), US EPA Pesticide Guidelines Subdivision F, reference No. 85-1 (1994). GLP compliant.

Bunn, H.F & Rosse, W. (2001) Hemolytic anemias and acute blood loss (Chapter 108). In: Braunwald, E., Fauci, A., Kasper, K.L., Hauser, S.L., Longo, D.L. & Jameson, I.L., eds, *Harrison's Principles of Internal Medicine*, 15th Ed., New York: McGraw-Hill, Medical Publishing Division.

Callander, R.D. (1995) Pirimicarb: an evaluation of the mutagenic potential using *S. typhimurium* and *E. coli*. Unpublished report No. CTL/P/4798 from Central Toxicology Laboratory, Zeneca. Conducted according to OECD guideline 471 and 472 (1983a, b). GLP compliant.

Callander, R.D. (2000a) Pirimicarb metabolite 062/06 (R031805): bacterial mutation assay in *S. typhimurium* and *E. coli*. Unpublished report No. CTL/YV4619/REG/REPT from Central Toxicology Laboratory; Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 471/472 (1983). GLP compliant.

Callander, R.D. (2000b) Pirimicarb metabolite 062/07 (R034865): bacterial mutation assay in *S. typhimurium* and *E. coli*. Unpublished report No. CTL/YV4620/REG/REPT from Central Toxicology Laboratory; Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 471/472 (1983). GLP compliant.

Chanarin, I. (1969) *The Megaloblastic Anaemias*. Oxford & Edinburgh: Blackwell Scientific Publications, pp. 40–63, 708–714.

Clapp, M.J., Garner, R. & Litchfield, M.H. (1972) Pirimicarb: 2-year feeding study in the rat. Unpublished report No. HO/IH/P/24 from ICI Ltd, Industrial Hygiene Research Laboratories.

Clay, P. (1996) Pirimicarb: L5178Y $TK^{+/-}$ mouse lymphoma mutation assay. Unpublished report No. CTL/P/5080 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 476 (1983). GLP compliant.

Clay, P. (2001a) Pirimicarb metabolite 062/06 (R031805): L5178Y $TK^{+/-}$ mouse lymphoma mutation assay. Unpublished report No: CTL/VV0225/REGULATORY/REPORT from Central Toxicology Laboratory. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 4736 (1997). GLP compliant.

Clay, P. (2001b) Pirimicarb metabolite 062/07 (R034865): L5178Y $TK^{+/-}$ mouse lymphoma mutation assay. Unpublished report No: CTL/VV0226/REGULATORY/REPORT from Central Toxicology Laboratory. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 476 (1997). GLP compliant.

Clay, P. (2001c) Pirimicarb metabolite 062/06 (R031805): *in vivo* rat liver unscheduled DNA synthesis assay. Unpublished report No: CTL/SR1104/REGULATORY/REPORT from Central Toxicology Laboratory. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 486 (1997). GLP compliant.

Clay, P. (2001d) Pirimicarb metabolite 062/06 (R031805): mouse bone marrow micronucleus test. Unpublished report No. CTL/SM1105/REGULATORY/REPORT from Central Toxicology Laboratory. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 474 (1997). GLP compliant.

Clapp, M.J., Garner, R. & Litchfield, M.H. (1972) Pirimicarb: 2-year feeding study in the rat. Unpublished report No. HO/IH/P/24 from ICI Central Toxicology Laboratory. Submitted to WHO by ICI Ltd.

Conning, D.M., Garner, R. & Griffiths, D. (1968) Ninety day oral toxicity of PP062—beagle dogs (Part 1). Unpublished report No. CTL/R/241 from ICI Ltd, Industrial Hygiene Research Laboratories. Submitted to WHO by ICI Ltd.

Conning, D.M., Garner, R. & Griffiths, D. (1969) Ninety day oral toxicity of PP062—beagle dogs (Part 2). Unpublished report No. CTL/R/248 from ICI Ltd, Industrial Hygiene Research Laboratories. Submitted to WHO by ICI Ltd.

Dacie, J.V. (1962) *The Haemolytic Anaemias, Part II. Auto-immune Haemolytic Anaemia*. London: J. & A. Churchill.

Dacie, J.V. (1967) *The Haemolytic Anaemias, Part III. Congenital and Acquired*. London: J. & A. Churchill.

Echobichon, D.J. (2001) Toxic effects of pesticides. In: Klaassen, C.D., ed., Casarett and Doll's Toxicology. 6th ed., New York, USA: Mc Graw Hill.

Ellman, G.L., Courtney, D.K., Andres, V. & Featherstone, R.M. (1961) A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochem. Pharmacol.*, **7**, 88–95.

Fletcher, K. (1971a) Pirimicarb (PP062): subacute oral toxicity of the plant metabolite, desmethylpirimicarb. Unpublished report No. HO/IH/T/842 from Industrial Hygiene Research Laboratories (now known as Central Toxicology Laboratory), Zeneca. Submitted to WHO by Syngenta Crop Protection AG.

Fletcher, K. (1971b) Pirimicarb (PP062): subacute oral toxicity of the plant metabolite, desmethylformamidopirimicarb. Unpublished report No. HO/IH/T/843 from Industrial Hygiene Research Laboratories (now known as Central Toxicology Laboratory), Zeneca. Submitted to WHO by Syngenta Crop Protection AG.

Fletcher, K. & Sotheran, J. (1971) Pirimicarb. 3-generation reproduction study in rat. Study No. CTL/R/339. Unpublished report from ICI Central Toxicology Laboratory. Submitted to WHO by ICI Ltd.

Flexner, J.M. & Hartmann, R.C. (1960): Megaloblastic anemia associated with anticonvulsant drugs. *Am. J. Med.*, **28**, 386–396.

Fox, T. (1978) Pirimicarb: dietary toxicity study in foxhounds. Unpublished report No. CTL/C/543 (Hazleton Report No. 1371-72/2) from Hazleton Laboratories, Europe Ltd, Harrogate, England. Submitted to WHO by Syngenta Crop Protection AG. Conducted before the guidelines and GLP.

Fox, V. (2001a) Pirimicarb metabolite 062/06 (R031805): *in vitro* cytogenetic assay in human lymphocytes. Unpublished report No. CTL/SV1030/REG/REPT from Central Toxicology Laboratory. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 473 (1997). GLP compliant.

Fox, V. (2001b) Pirimicarb metabolite 062/07 (R34865): *in vitro* cytogenetic assay in human lymphocytes. Unpublished report No. CTL/SV1031/REG/REPT from Central Toxicology Laboratory. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 473 (1997). GLP compliant.

Garner, R. (1972) Pirimicarb anaemia (a chemically-induced autoimmune haemolytic anaemia). Unpublished report No CTl/P/61 from ICI Ltd, Industrial Hygiene Research Laboratories.

Garner, R., Conning, D.M. & Hermoso-Perez, C. (1967) Factor VII deficiency in beagle dog plasma and its use in the assay of the human factor VII. *Nature* (*Lond.*) **216**, 1130–1132.

Garner, R. & Smith, •• (1972) Pirimicarb: 2-year feeding study in the rat. Unpublished report No. CTL/P/24 from ICI Ltd, Industrial Hygiene Research Laboratories.

Garner, R., Phillips, C.E. & Slack, P. (Report revised in 1995 but originally issued in 1972) First revision to pirimicarb (PP062): pirimicarb anaemia (a chemically-induced autoimmune haemolytic anaemia). Unpublished report No. CTL/P/61 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. No guidelines applicable. Conducted before GLP.

Gledhill, A.J. (1998) Pirimicarb: biotransformation in the rat. Unpublished report No. CTL/P/5530 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 417 (1984), 87/302/EEC B.36 (1987), Agricultural Chemical Laws & Regulations, Japan (1985), US EPA Pesticide Guidelines Subdivision F, reference No. 85-1 (1994). GLP compliant.

Griffiths, D. & Conning, D.M. (Report revised in 1995 but originally issued in 1968) First revision to ninety-day oral toxicity of PP062—albino rats. Unpublished report No. CTL/R/237 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conduct consistent with 87/302/EEC B.26 and OECD 408 (1981), but before GLP.

Harris, J.W. (1954) Studies on the mechanism of a drug-induced haemolytic anemia. *J. Lab. Clin. Med.*, **44**, 809–810 (Abstract 73).

Hodge, M.C.E. (1989) Pirimicarb: teratogenicity study in the rat. Unpublished report No. CTL/P/2745 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 414 (1981).

Hodge, M.C.E. (1995a) Subchronic studies with pirimicarb (PP062) in the beagle dog: Ninety-day oral toxicity of PP062—beagle dogs (Part 1) and oral toxicity of PP062—beagle dogs (Part 2). Unpublished report No. CTL/P/4593 incorporating CTL/R/241 and CTL/R/248 (previously IHR/241 and IHR/248) from Central Toxicology Laboratory (formerly known as Industrial Hygiene Research Laboratories), Zeneca. Submitted to WHO by Syngenta Crop Protection AG.

Hodge, M.C.E. (1995b) First revision to pirimicarb (PP062): 2-year feeding study in beagle dogs'. Unpublished report No. CTL/R/337 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 452 (1981), 67/548/EEC B.30, US EPA Pesticide Guidelines Subdivision F, reference No. 83-1 (1994).

Horner, S.A. (1996a) Pirimicarb: acute neurotoxicity study in rats. Unpublished report No. CTL/P/5232 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to FIFRA § 82–88.

Horner, S.A. (1996b) Pirimicarb: subchronic neurotoxicity study in rats. Unpublished report No. CTL/P/5233 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to FIFRA § 81–87.

Horner, S.A. (1998) Pirimicarb: 1-year oral toxicity study in dogs. Unpublished report No. CTL/P/5690 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 452 (1981), 87/302/EEC OJ L133 (1988), US EPA Pesticide Guidelines Subdivision F, reference No. 83-1 (1994). GLP compliant.

Huennekens, F.M. (1966) Biochemical functions and interrelationships of folic acid and vitamin B12. *Prog. Hematol.*, **5**, 83–104.

Jones, K. & Howard, C.A. (1989) Pirimicarb (technical): an evaluation in the mouse micronucleus test. Unpublished report No. CTL/P/2641 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 474 (1983). GLP compliant.

Kennelly, J.C. (1990) Pirimicarb: assessment for the induction of unscheduled DNA synthesis in rat hepatocytes *in vivo*. Unpublished report No. CTL/P/2824 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 486 (1983). GLP compliant.

Kidd, P. & Mollin, D.L. (1957) Megaloblastic anemia and vitamin-BI2 deficiency after anticonvulsant therapy: report of two cases, *BMJ,* **ii**, 974–976.

Lees, D. (2001) Pirimicarb metabolite 062/06 (R031805): 90-day dietary toxicity study in rats. Unpublished report No. CTL/ PR1184 from Syngenta UK. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 408 (1981), 87/302/EEC B.26, US EPA OPPTS 870.3100. GLP compliant.

Lees, D. & Connolly, H.J. (1995a) Pirimicarb: acute oral toxicity to the rat. Unpublished report No. CTL/P/4802 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. GLP compliant.

Lees, D. & Connolly, H.J. (1995b) Pirimicarb: acute dermal toxicity to the rat. Unpublished report No. CTL/P/4855 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. GLP compliant.

Lees, D. & Connolly, H.J. (1995c) Pirimicarb: skin irritation to the rabbit. Unpublished report No. CTL/P/4858 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. GLP compliant.

Lees, D. & Doyle, C.E. (1995) Pirimicarb: eye irritation to the rabbit. Unpublished report No. CTL/P/4706 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. GLP compliant.

Lees, D. & Leah, A.M. (1995) Pirimicarb: 21-day dermal toxicity to the rat. Unpublished report No. CTL/P/4805 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 410 (1981), 92/69/EEC B.9. GLP compliant.

Lefevre, V.K. & Parkinson, G.R. (1974) Pirimicarb (PP062; 2-dimethylamino-5,6-dimethylpyrimidin-4-yldimethylcarbamate): acute oral toxicities of plant and mammalian metabolites. Unpublished report No. CTL/P/101B from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted before GLP.

Lotti, M. (2000) Organophosphorus compounds. In: Spencer, P.S. & Schaumburg, H.H., eds, *Experimental and clinical neurotoxicology*, 2nd Ed., New York, USA: Oxford University Press.

McGregor, D.B. (1974) Dominant lethal study in mice of ICI PP062. Zeneca unpublished report No. CTL/C/256 from Inveresk Research International. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 478 (1983). Conducted before GLP.

MacIntosh, P.C. & Hutchinson, J.L. (1960) Megaloblastic anaemia due to anticonvulsant therapy: report of a case responding to vitamin B12. *Can. Med. Ass. J.*, **82**, 365–368.

Magnusson, B. & Kligman, A.M. (1970) *Allergic contact dermatitis in the guinea pig. Identification of contact allergens*. Springfield, Illinois: Charles C. Thomas.

Milburn, G.M. (1989) Pirimicarb: teratogenicity study in the rabbit. Unpublished report No. CTL/P/2680 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD 414 (1981).

Moxon, M.E. (1991) Pirimicarb: multigeneration study in the rat. Unpublished report No. CTL/P/2940 Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 416 (1983). GLP compliant.

Palmer, S.M. & Samuels, D.M. (1974) Pirimicarb: 80-week carcinogenic study in mice. CTL Study No. HO/CTL/P121/B. Submitted to WHO by ICI.

Parkinson, G.R. (1974) Pirimicarb metabolites (R31680 and R35140): acute oral toxicity. Unpublished report No. HO/CTL/P/144 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG.

Parkinson, G.R. (1975) Oral toxicities of three guanidine compounds. Unpublished Report No. CTL/P/160 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG.

Parkinson, G.R. (1978) Pirimicarb metabolite R34885: subacute oral toxicity to rats. Unpublished report No. CTL/P/402 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG.

Parkinson, G.R. (Report revised in 1979 but originally issued in 1978) Pirimicarb metabolite R34836: subacute oral toxicity to rats. Unpublished report No. CTL/P/401 (revised) from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG.

Parr-Dobrzanski, R.J. (1994) Pirimicarb: 4-hour acute inhalation toxicity study in the rat. Unpublished report No. CTL/P/4522 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. GLP compliant.

Paul, D., Richards, D., Banham, P.B. & Weight, T.M. (Original report date: 1978; revised by Hodge, M.C.E, 1995) First revision to pirimicarb: growth study to determine a no-effect level in the female rat. Unpublished report No. CTL/P/408 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG.

Rattray, N.J. (1998) Pirimicarb: 80-week carcinogenicity study in mice. Unpublished report No. CTL/P/5839 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 451 (1983). GLP compliant.

Rattray, N. & Leah, A.M. (1990) Pirimicarb: skin sensitisation to the guinea pig. Unpublished report No. CTL/P/3087 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. GLP compliant.

Reynolds, E.H, Hallpike, J.F., Phillips, B.M. & Matthews, D.M. (1965) Reversible absorptive defects in anticonvulsant megaloblastic anaemia. *J. Clin. Path.*, **18**, 593–598.

Richards, D., Banham, P.B. & Weight, T.M. (1978) Pirimicarb: paired feeding study in the female rat. Unpublished report No. CTL/P/407 Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG.

Sotheran, M.F., Banham, P.B., Jackson, D.G., Taylor, K., Weight, T.M. & Woollen, B.H. (1980) Pirimicarb: lifetime feeding study in the mouse. Unpublished report No. CTL/P/0491 from Central Toxicology

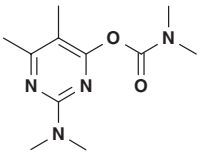
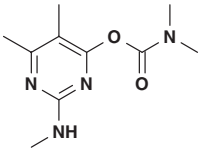
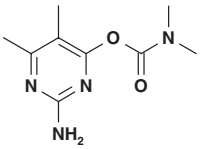
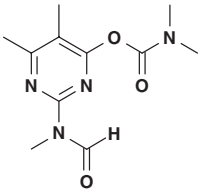
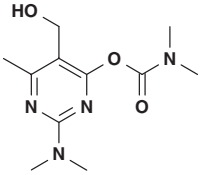
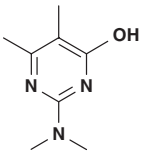
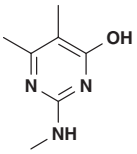
Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted before OECD guidelines and GLP.

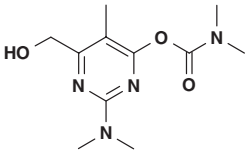
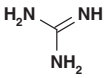
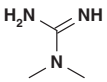
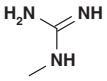
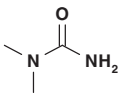
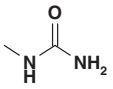
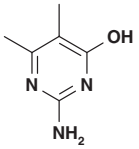
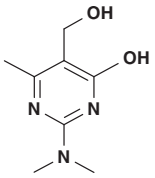
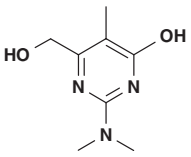
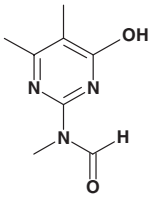
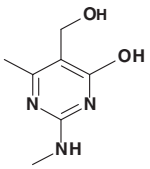
Tinston, D.J. (1992) Pirimicarb: Two-year feeding study in rats. Unpublished report No. CTL/P/3040 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 453 (1983). GLP compliant.

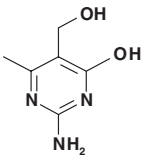
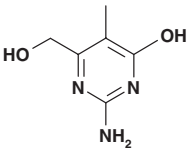
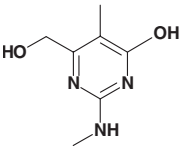
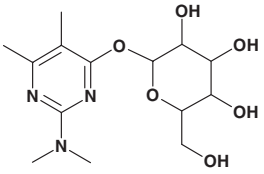
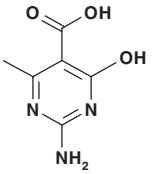
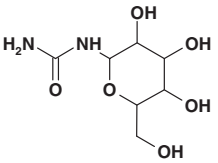
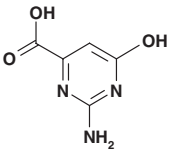
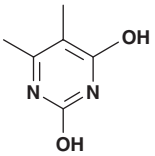
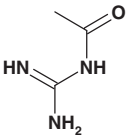
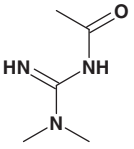
Trueman, R.W. (1980) An examination of pirimicarb for potential mutagenicity using the Salmonella/microsome reverse mutation assay. Unpublished report No. CTL/P/540 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted to a protocol that was consistent with the OECD guideline 471 (1983). GLP compliant.

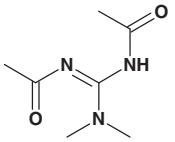
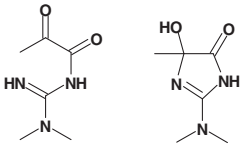
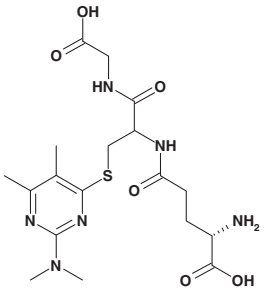
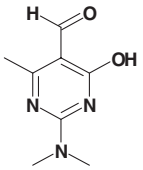
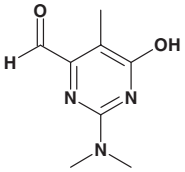
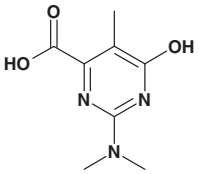
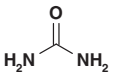
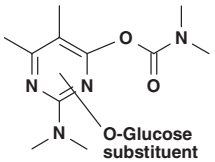
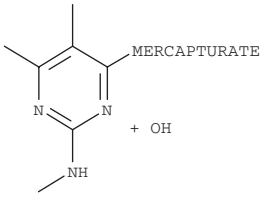
Wildgoose, J., Howard, C.A., Richardson, C.R. & Randall, V. (1987) Pirimicarb: a cytogenetic study in human lymphocytes *in vitro*. Unpublished report No. CTL/P/1655 from Central Toxicology Laboratory, Zeneca. Submitted to WHO by Syngenta Crop Protection AG. Conducted according to OECD guideline 473 (1983). GLP compliant.

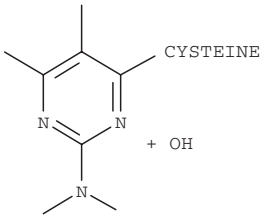
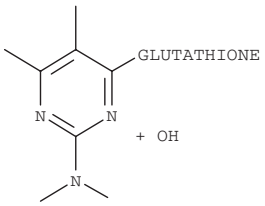
APPENDIX

Compound name	IUPAC name	Structure	Study in which metabolite was identified
Pirimicarb 062/01 R32062 I X (as O-Glucuronide)	2-dimethylamino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate		Parent active ingredient Primary crops—lettuce, potato, apple, wheat Crop rotation Rat biotransformation
836/01 Desmethyl pirimicarb R34836 III XIII (as *O*-Glucuronide)	5,6-dimethyl-2-(methylamino) pyrimidin-4-yl dimethylcarbamate		Primary crops—lettuce, potato, apple, wheat Crop rotation Soil aerobic degradation Soil anaerobic degradation Soil surface photolysis Field soil dissipation Aqueous photolysis Water sediment Rat biotransformation
062/03 R35140 IV XII	2-amino-5,6-dimethylpyrimidin-4-yl dimethylcarbamate		Primary crops—lettuce Crop rotation Field soil dissipation Aqueous photolysis Rat biotransformation
062/04 Desmethylformamido pirimicarb R34885 II	5,6-dimethyl-2-(methylformamido)pyrimidin-4-yl dimethylcarbamate		Primary crops—lettuce, potato, apple, wheat Crop rotation Soil aerobic degradation Soil anaerobic degradation Soil surface photolysis Field soil dissipation Aqueous photolysis Water sediment
062/05 R238359	2-dimethylamino-5-hydroxymethyl-6-methylpyrimidin-4-yl dimethylcarbamate		Tentative—primary crop—apple
062/06 R31805 V III (rat as O-glucuronide conjugate) IV (rat as free metabolite)	2-dimethylamino-5,6-dimethylpyrimidin-4-ol		Primary crops—lettuce, potato, apple, wheat Crop rotation Livestock—hen, goat Soil aerobic degradation Soil anaerobic degradation Field soil dissipation Aqueous photolysis Rat biotransformation
062/07 R34865 VI VIII (as O-glucuronide conjugate)	5,6-dimethyl-2-(methylamino) pyrimidin-4-ol		Primary crops—lettuce, apple Crop rotation Livestock—hen, goat Soil aerobic degradation Soil anaerobic degradation Field soil dissipation

Compound name	IUPAC name	Structure	Study in which metabolite was identified
062/08 R238177	2-dimethylamino-6-hydroxymethyl-5-methylpyrimidin-4-yl dimethylcarbamate		Primary crops—wheat, lettuce
062/09 Guanidine R12378 X	Guanidine		Crop rotation Tentative—wheat primary crop
062/10 1,1-Dimethylguanidine R16210 VIII	1,1-Dimethylguanidine		Primary crops—potato, apple, wheat Soil surface photolysis Aqueous photolysis
062/11 Methylguanidine R16192 IX	1-Methylguanidine		Primary crops—potato, wheat Soil surface photolysis
062/12 R7272	1,1-Dimethylurea		Not detected
062/13 R57659	Methylurea		Not detected
062/14 R31680 VII II	2-Amino-5,6-dimethylpyrimidin-4-ol		Tentative—lettuce primary crop Livestock—hen, goat Field soil dissipation Rat biotransformation
062/15 R404094	2-Dimethylamino-5-hydroxymethyl-6-methylpyrimidin-4-ol		Tentative—lettuce primary crop Tentative—apple primary crop
062/16 R404137	2-Dimethylamino-6-hydroxymethyl-5-methylpyrimidin-4-ol		Tentative—apple primary crop
062/17 R407392	2-(*N*-methylformamido)-5,6-dimethylpyrimidin-4-ol		Tentative—lettuce primary crop
062/18 R406405	5-Hydroxymethyl-6-methyl-2-(methylamino)pyrimidin-4-ol		Tentative—lettuce primary crop Tentative—milk from goat study

Compound name	IUPAC name	Structure	Study in which metabolite was identified
062/19 R407135	2-Amino-5-hydroxymethyl-6-methylpyrimidin-4-ol		Not detected
062/20 R409239	2-Amino-6-hydroxymethyl-5-methylpyrimidin-4-ol		Not detected
062/21 R409238 V (rat)	6-Hydroxymethyl-5-methyl-2-(methylamino)pyrimidin-4-ol		Tentative—lettuce crop metabolism Rat biotransformation
062/22 R409464	2-Dimethylamino-5,6-dimethyl-4-(β-D-glucos-6-yl)pyrimidine		Not detected
062/23 R4715	2-Amino-4-hydroxy-6-methylpyrimidin-5-carboxylic acid		Not detected
062/24 R26021	β-d-Glucosylurea		Not detected
062/25 R59480	2-Amino-6-hydroxypyrimidin-4-carboxylic acid		Not detected
062/26 R99366	5,6-Dimethylpyrimidin-2,4-diol		Not detected
062/27 R16229	1-Acetylguanidine		Not detected
062/28 R32379	1-Acetyl-3,3-dimethylguanidine		Soil surface photolysis

Compound name	IUPAC name	Structure	Study in which metabolite was identified
062/29 R411934	2,3-Diacetyl-1,1-dimethylguanidine		Soil surface photolysis
062/30 R411893	3,3-Dimethyl-1-(2-oxopropionyl) guanidine		Not detected
062/31 R413303	(*r*)-3-Aza-5-glutamino-4-oxo-6-(2-dimethylamino-5,6-ylthio)hexanoic acid		Not detected
062/32 R35251	2-Dimethylamino-4-hydroxy-6-methylpyrimidin-5-al		Not detected
062/33 R414656	2-Dimethylamino-6-hydroxy-5-methylpyrimidin-4-al		Not detected
062/34 R414657	2-Dimethylamino-6-hydroxy-5-methylpyrimidin-4-carboxylic acid		Not detected
Urea R1498	Urea		Primary crop—apple
Metabolite A			Crop rotation
VI			Rat biotransformation

Compound name	IUPAC name	Structure	Study in which metabolite was identified
IX	4-Cysteinyl-hydroxymethyl-2-dimethylmethylamino-pyrimidine		Rat biotransformation
XIV			Rat biotransformation

PROPICONAZOLE

First draft prepared by
I. Dewhurst[1] and V. Dellarco[2]
[1] Pesticides Safety Directorate, Department for Environment, Food and Rural Affairs, Mallard House, Kings Pool, York, England; and
[2] United States Environmental Protection Agency, Office of Pesticide Programs, Health Effects Division, Washington DC, USA

Explanation

Propiconazole is the International Organization for Standardization (ISO) approved name for 1-[2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl-methyl]-1*H*-1,2,4-triazole, a systemic fungicide that acts by inhibition of ergosterol biosynthesis. Propiconazole was evaluated toxicologically by the JMPR in 1987, when an acceptable daily intake (ADI) of 0–0.04 mg/kg bw was established on the basis of the no-observed-adverse-effect level (NOAEL) of 4 mg/kg bw per day for effects on body weight, clinical chemistry and haematology in a 2-year study in rats, and this was supported by the NOAEL of 7 mg/kg bw per day in a 1-year study in dogs. Propiconazole was considered by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues. The Meeting reviewed new data on propiconazole that had not been reviewed previously and relevant data from the previous evaluation.

Evaluation for acceptable daily intake

Most of the studies of toxicity with propiconazole were performed between 1979 and 1988 and used batches with a purity of 89–93%, which is considered to be representative of commercial propiconazole. Studies did not comply with good laboratory practice (GLP) unless specifically stated in the text.

1. Biochemical aspects

1.1 Absorption, distribution and excretion

(a) Oral route

Mice

Groups of five male and five female CD1 mice were fed diets containing propiconazole at a concentration of 5, 100 or 2500 ppm for 21 days, followed by a single oral dose of [U-^{14}C]-phenyl propiconazole (specific activity, 2.1 MBq/mg; radiochemical purity, >97%) at a corresponding dose (0.8/1.0, 16.8/21.5 and 434/475 mg/kg bw for males/females). Additional groups of two female mice and two male RAIf rats received control diet and radiolabelled propiconazole as a single dose at 600 mg/kg bw or 9 mg/kg bw respectively. The radiolabelled propiconazole was administered by gavage in a vehicle of ethanol/polyethylene glycol/water (7 : 9 : 4). At the higher dose, unlabelled propiconazole (purity, 91.1%) was added to the radiolabelled preparation. Urine and faeces were collected at 24-h intervals. All animals were sacrificed 4 days after administration of the final dose and tissue concentration of radioactivity was determined by liquid scintillation counting (LSC) after appropriate processing of the sample.

Two mice in the control group died after receiving a dose of 600 mg/kg bw by gavage. Total recoveries of radioactivity were >85% of the administered dose. Urinary excretion occurred predominantly in the first 24 h and tended to be higher in males than in females (Table 1). Residual radioactivity was approximately proportional to the dose administered. Residues in female mice were higher than in male mice, except in the kidneys where values were higher or equal in males. Independent of the dose and sex of the animals, the highest concentrations of residues (up to 3.0 µg/g) were found in the liver (Bissig, 1986).

Table 1. Excretion patterns (% of administered dose) in mice pretreated with diets containing unlabelled propiconazole for 21 days before administration of a single dose of radiolabelled propiconazole by gavage

Sample	Dietary concentration (ppm)/^{14}C-labelled dose (mg/kg bw)					
	5/0.8	100/17	2500/434	5/1	100/22	2500/475
	Males			Females		
Faeces						
0–24 h	35	33	26	35	15	20
0–96 h	38	35	32	43	22	31
Urine						
0–24 h	47	58	57	39	71	44
0–96 h	54	60	67	45	81	52
Total excretion	91	95	99	87	102	83

From Bissig (1986)

Rats

Two male and two female rats were given single doses of triazole-labelled [3,5-^{14}C]propiconazole (radiochemical purity, >98%) at 0.5 or 25 mg/kg bw by gavage in ethanol/polyethylene glycol 200/water (3 : 2 : 5). Within 24 h, 74–84% of the administered radioactivity had been excreted. After 6 days, 0.04–0.15%, 28–46% and 53–67% of the radioactivity had been recovered from expired air, faeces and urine respectively. Total recoveries of radioactivity were >95%. The patterns of excretion were essentially independent of sex or dose, although there was an indication that females excreted more propiconazole at the lower dose via the urine. Less than 1% of the administered dose remained in the tissues. Highest tissue concentrations of residues at day 6 were found in the liver, blood and kidneys, all <1 µg equivalent/g. No unchanged propiconazole was excreted in the urine according to thin-layer chromatography (TLC) analyses (Hambock, 1979).

A single oral dose of triazole-labelled [3,5-^{14}C]propiconazole (specific activity, 1.44 MBq/mg; radiochemical purity, >98%) at about 32 mg/kg bw or of phenyl-[U-^{14}C]-labelled propiconazole (specific activity, 2.22 MBq/mg; radiochemical purity, >98%) was administered to groups of male Tif:RAIf rats (20 rats were given triazole-labelled propiconazole; three rats were given for phenyl-labelled propiconazole) via gavage. More than 80% of the administered radioactivity was excreted within 24 h, with an approximately even distribution between the urine and faeces (Table 2). There was no marked difference in excretion of propiconazole labelled on either of the two positions (Mücke, 1979).

The most extensive investigation of the toxicokinetics of propiconazole was performed in a GLP-compliant study (Cresswell & Hopkins, 1989). Groups of five male and five female Crl:CD(SD)BR rats received [U-^{14}C]phenyl propiconazole (specific activity, 1.44 MBq/mg; radiochemical purity, >99%) as a single dose at 0.5 or 50 mg/kg bw by gavage after a 16-h fast, or as a single dose at 0.5 mg/kg bw by intravenous administration. The lower dose was given either with no pretreatment or after 14 unlabelled doses. Vehicles were physiological saline for intravenous dosing and ethanol/polyethylene glycol 200/water (1 : 2 : 2) for gavage. All animals were sacrificed after 168 h, except for animals in the pretreated group, which were sacrificed after 120 h). Samples of urine and faeces were collected over nine intervals of up to 168 h. Exhaled carbon dioxide was collected during the first 24 h. At termination, samples from a range of tissue and blood were removed. Samples of excreta and tissues were assayed by LSC after appropriate processing.

Some animals lost weight during the study, but there were no clinical signs indicating toxicity. Adequate stability and content of the dosing solutions was demonstrated. No carbon dioxide was detected in samples of trapped air. Patterns of excretion were similar

Table 2. Excretion of radioactivity (% of administered dose) in male rats given a single dose of phenyl- or triazole-labelled propiconazole

	Triazole-[3,5-^{14}C]-labelled propiconazole at 31.4 mg/kg bw	Phenyl-[U-^{14}C]-labelled propiconazole at 32.5 mg/kg bw
Urine		
0–24 h	44.5	48.5
0–96 h	52.3	51.4
Faeces		
0–24 h	36.2	44.2
0–96 h	43.3	48.6

From Mücke (1979)

Table 3. Radioactivity (mean values) in samples of excreta and tissues from rats given [U-^{14}C]phenyl propiconazole by intravenous or oral administration

	Dose (mg/kg bw)							
	0.5	0.5	0.5 (14 + 1)[a]	50	0.5	0.5	0.5 (14 + 1)[a]	50
	IV	Gavage	Gavage	Gavage	IV	Gavage	Gavage	Gavage
	Males				Females			
Faeces								
0–24 h (%)	35	37	34	31	30	32	31	27
0–168 h (%)	42	50	48[b]	48	39	38	40[b]	37
Urine								
0–24 h (%)	40	36	36	34	44	43	43	46
0–168 h (%)	43	39	41[b]	39	46	44	46[b]	49
Liver (ppm)	0.021	0.012	0.022	0.94	0.01	0.007	0.018	0.78
Kidney (ppm)	0.006	0.004	0.006	0.35	0.005	0.004	0.007	0.37
Lung (ppm)	0.003	ND	0.001	0.08	ND	0.001	0.003	0.08
Adrenal (ppm)	0.003	ND	0.002	0.56	0.006	ND	0.01	0.26
Blood (ppm)	0.001	ND	ND	0.07	0.004	ND	0.002	0.16
Bone marrow (ppm)	0.05	ND	ND	ND	0.008	0.006	0.04	0.14
Total recovery (%)	86–92	92–102	95–98	88–99	80–108	90–98	93–102	89–97

From Cresswell & Hopkins (1989)
IV, intravenous; ND, not detected
[a] This dose was administered after 14 unlabelled doses.
[b] 120 h

in all groups irrespective of dosing regime or route of administration, with most of the administered dose being excreted within 24 h and an approximately equal distribution between faeces and urine (Table 3). Females tended to excrete a greater proportion of an oral dose in the urine than did males (Table 3). Intragroup variation in patterns of excretion was low. Concentrations of radioactivity remaining at the end of the study were low (≤1% of the administered dose), most tissue concentrations were <0.1 ppm (µg equivalents/g) with highest concentrations typically in the liver and kidney (Table 3). Radioactivity in bone marrow showed a high degree of intra- and intergroup variation. The similarity in urinary and faecal excretion patterns between oral and intravenous dosing indicated significant biliary excretion and that most of an oral dose is absorbed (Cresswell & Hopkins, 1989).

The absorption, distribution and excretion of [U-^{14}C]phenyl propiconazole (specific activity, 1.48 MBq/mg; radiochemical purity, ≥ 98%) was investigated in intact and bile-duct cannulated Tif:RAIf male rats. A single dose at 0.5 mg/kg bw was administered by gavage in ethanol/polyethylene glycol 200/water (1 : 2 : 2). Blood samples were collected, at regular intervals up to 48 h, from the retro-orbital plexus of three rats. Groups of six rats were sacrificed at 1, 8, 14 or 24 h for investigation of radioactivity in a range of tissues. Samples of urine, faeces (0–24 and 24–48 h) and bile (eight intervals during 48 h) were collected from five bile-duct cannulated rats.

Peak plasma concentrations of radioactivity were detected 1 h after dosing. Highest tissue concentrations were found in the liver and kidney (Table 4). Most of the administered dose was excreted within 24 h in the bile (58%), with smaller amounts in the urine (15%) and faeces (4%); total excretion during 48 h was >90%. When compared with the results of studies in intact animals in which about 50% of the administered dose was recovered in the urine, these findings indicate extensive enterohepatic recirculation. Taking into account both biliary and urinary components, these results indicate that >80% of an oral dose of propiconazole is absorbed (Bissig, 1992).

Table 4. Tissue concentrations of radioactivity (μg equivalents/g) in male rats given [U-^{14}C]phenyl propiconazole as a single dose at 0.5 mg/kg bw

Tissue	Time after dosing (h)			
	1	8	14	20
Liver	0.68	0.58	0.15	0.14
Kidney	0.25	0.23	0.07	0.08
Lung	0.11	0.10	0.05	0.03
Adrenal gland	0.14	0.11	0.03	0.03
Plasma	0.08	0.07	0.02	0.02

From Bissig (1992)

Table 5. Total radioactivity and levels of main metabolites in samples from Leghorn hens given 10 mg of [U-^{14}C]phenyl propiconazole per day for 8 days

Sample	Mean total radioactivity (ppm)	Metabolite (mean % of radioactivity in sample)		
		Propiconazole	CGA 118244	CGA91305
Liver	3.9	2	4	81
Kidney	4.2	2	2	47
Egg white	0.7	27	51	18
Egg yolk	1.7	15	11	62
Thigh muscle	0.6	7	2	80
Skin/fat	0.6	40	3	30

From Doweyko (1990a)
CGA 118244, (propyl) β-hydroxy-propiconazole
CGA 91305, hydroxy-de-dioxolanated propiconazole

Hens

Two Leghorn hens were given daily doses of gelatin capsules containing either 5.9 mg of [^{14}C]phenyl propiconazole or 5.5 mg of [^{14}C]triazole propiconazole, each day for 16 days. These doses equated to a dietary concentration of approximately 50 ppm. The hens were sacrificed 24 h after the last dose. Concentrations of residues in eggs, excreta and tissues were determined. More than 94% of the administered doses were found in excreta, with <1% remaining in eggs/tissues. The concentrations of residue in the egg white of the hen receiving phenyl-labelled propiconazole were unusual, declining to day 10 and then increasing markedly. Residues of the triazole label in eggs reached a plateau after 11 doses. Peak concentrations in eggs were 0.9 to 1.2 ppm, highest tissue concentrations were approximately 2 ppm in the liver and kidney. Concentrations of radioactivity were generally higher in eggs and tissues (especially muscle) from the animal receiving the triazole-labelled propiconazole, indicating cleavage of the link between the triazole and phenyl rings (Seim & Brown, 1984; Szolics & Simoneaux, 1985).

Four Leghorn hens received daily doses of 10 mg of [U-^{14}C]phenyl propiconazole (specific activity, 1.77 MBq/mg; radiochemical purity, 98.6%) in gelatin capsules for 8 days, before sacrifice 6 h after the final dose. Steady-state excretion accounted for approximately 80–85% of the administered dose. Concentration of radioactivity in tissues and eggs was determined by LSC after appropriate processing. Highest tissue concentrations were found in the liver and kidney (Table 5). Concentrations of radioactivity in eggs reached a plateau by days 5–7 (Table 5) (Doweyko, 1990a).

Goats

One lactating goat received 10 daily doses of 5 mg of triazole-labelled propiconazole (specific activity, 0.92 MBq/mg) equivalent to a dietary concentration of 4.5 ppm. Samples of milk, urine and faeces were taken during the study. The animal was sacrificed 27 h after the final dose and a range of tissues were removed. Total radioactivity was determined by LSC after sample processing. Most of the administered doses were excreted in the urine (70%), with 20% in the faeces and approximately 0.2% in the milk. Concentrations in milk reached a plateau at approximately 0.01–0.02 ppm after six doses, with most radioactivity being found in the whey (80%) and casein (17%), and approximately 1% in the fat. Peak tissue concentrations were found in the liver (0.1 ppm) and kidney (0.03 ppm), with 0.01 ppm in muscle (Fisher & Cassidy, 1980)

Two lactating goats were given four daily doses of 125 mg of [U-^{14}C]phenyl propiconazole (specific activity, 1.77 MBq/mg; radiochemical purity, 98.6%) in gelatin capsules. The doses equated to dietary concentrations of 67–92 ppm. Samples of urine, faeces and milk were collected throughout this GLP-compliant study. The goats were sacrificed 6 h after the final dose and samples of a range of tissues were taken. Samples of excreta, milk and tissue were analysed by LSC for total radioactivity. Excretion was extensive in the urine (50% of the administered dose) and faeces (40%) but not in milk. Tissue concentrations were highest in the liver (4 ppm) and kidney (2.5 ppm), with lower concentrations in blood (0.3 ppm). The highest concentration in milk (0.2 ppm) was seen on day 4 and it is possible that a plateau had not been reached (Doweyko, 1990b).

(b) Dermal route

Rats

Groups of four male and four female Sprague-Dawley rats were given triazole-U-^{14}C-propiconazole as single doses at 1.0 or 10.0 mg/kg bw as a 40% solution in a formulation blank applied to the dorsal skin. The application site did not appear to be covered and oral ingestion cannot be discounted. Extensive absorption was indicated, with 20% of the applied dose being excreted within 24 h and >75% of the applied radioactivity being recovered during 72 h in excreta (urine, 30–40%; faeces, 20–30%) or the tissues and carcass other than at the application site. The amount of residual radioactivity on the skin averaged 20% of the applied dose. Highest concentrations in tissues were generally found at 24 h, although females at the lower dose and males at the higher dose appeared to have an initial peak at 4 h. The report states that the metabolites in excreta were similar to those formed in animals dosed orally (no data presented) (Simoneaux, 1983).

Comparable results were reported by Murphy et al. (1986) in a study in which groups of four male Sprague-Dawley rats were given triazole-^{14}C-labelled propiconazole (radiochemical purity, >95%) at a dose of 0.1, 1 or 10 mg per rat by dermal administration in a formulation blank. Animals were sacrificed after 2, 4 or 10 h. Total recoveries of radioactivity were >85%. The extent of absorption increased with time, but was inversely proportional to applied dose. After 10 h, total absorption (excluding skin residue) was approximately 30%, 10% and 5% for the lowest, intermediate and highest dose respectively. Approximately 25% of the intermediate and highest doses, and 13% of the lowest dose remained at the application site after washing (Murphy et al., 1986).

1.2 Biotransformation

Mice

Samples of urine from the study of Bissig (1986), in which [U-^{14}C]phenyl-propiconazole was used (described above), were investigated using TLC, high-performance liquid chromatography (HPLC), nuclear magnetic resonance (NMR) and mass spectrometry (MS). The presence of conjugates was examined using glucuronidase or sulfatase treatments. The pattern of urinary metabolites of propiconazole demonstrated a marked sex dependency. In male mice, 60% of the radioactivity in urine collected at 0–24 h was represented by one metabolite (U2), while this metabolite accounted for only 30% in the sample of urine collected at 0–24 h in female mice and aproximately 10% in rats. U2 was identified as the glucuronic acid conjugate of 1-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)ethanol—a metabolite lacking the dioxolane ring substituent. Treatment of U2 with glucuronidase resulted in a significant increase in levels of U16 in mice, but little change in rats, indicating that it might consist of more than one component. Other major metabolites (U1 and U12; not identified) were present in greater amounts in females than in males. The results indicate that the major metabolic pathway of propiconazole in mice involves dioxolane ring cleavage followed by glucuronide conjugation (Bissig, 1986).

Rats

Samples of urine and faeces from animals receiving triazole-labelled propiconazole in the study by Mücke (1979), described above, were investigated using glucuronidase/arylsulfatase digestion and two-dimensional TLC and electrophoresis. Faecal metabolites were less polar than those in the urine. Approximately 20 metabolites were found in the urine. Unchanged propiconazole was present at approximately 5% in the faecal samples, but was not detected in urine. Conjugates represented approximately 10% of the administered dose. The primary urinary metabolite (U8) represented 24% of the urinary radioactivity. The structure of propiconazole was reported to be well preserved, with little cleavage of the dioxolane ring (Mücke, 1979).

The metabolism of [3,5-^{14}C]-triazole propiconazole (specific activity 23.1 µCi/mg (0.85 MBq/mg); radiochemical purity >98%) was investigated in male TIF:RAIf rats (group size not specified). Rats were fasted overnight before being given a dose of approximately 31.4 mg/kg bw by gavage in an ethanol/polyethylene glycol 200/water (3 : 2 : 5) vehicle. Samples of urine and faeces were taken during the first 24 h after dosing; these represented 45% and 36%, respectively, of the administered radioactivity. Samples were extracted and investigated using a wide range of techniques, including fractionation, derivitization, enzymic hydrolysis, TLC, electrophoresis, MS, HPLC, liquid chromatography (LC) and NMR. Nine reference compounds were used for confirmation of identity. Propiconazole was not found in urine, but was present in faeces at approximately 3% of the administered dose. A wide range of metabolites were identified (representing more than 50% of the administered radioactivity), most being present at <2% of the administered dose. The major site of metabolism was the propyl side-chain, with approximately 15–20% of the radioactivity consisting of carboxy acid and or hydroxy derivatives from the propyl side-chain. Dioxolane ring cleavage was evident in the urinary metabolites, representing 10–15% of the administered dose. Hydroxylation of both chlorophenyl and triazole rings was seen together with glucuronidation and sulfation. There was limited evidence of cleavage between the chlorophenyl and triazole rings (Mücke, 1983).

Pooled samples of urine and pooled samples of faeces from the study by Cresswell & Hopkins (1989) (see above), were extracted and analysed for metabolites by two-dimensional TLC. There was no investigation of conjugation. Comparisons were made with eight analytical standards of predicted metabolites. Propiconazole was found to be extensively metabolized; however, many of the components did not correlate with the standards (Table 6). Twenty-four urinary and forty-seven faecal components were detected among the range of samples, with great variation between the patterns for the sexes and at different doses (Table 6). It was not possible to compare faecal and urinary patterns directly, as different first-solvent systems were used. Unchanged propiconazole was present in the faeces of orally dosed groups (6–18% of the administered radioactivity) and in the urine of animals treated by intravenous administration (approximately 30% of the administered radioactivity). With many metabolites remaining unidentified, the metabolic pathway of propiconazole could not be determined with any certainty. The levels of CGA 91304 in pretreated

Table 6. Urinary and faecal metabolites (% of radioactivity of fraction) in samples from rats given [U-^{14}C]phenyl propiconazole by oral or intravenous administration

	Dose (mg/kg bw)							
	0.5	0.5	0.5 (14 + 1)[a]	50	0.5	0.5	0.5 (14 + 1)[a]	50
	IV	Gavage	Gavage	Gavage	IV	Gavage	Gavage	Gavage
	Males				Females			
Urine								
Propiconazole	27	—	—	—	29	—	—	—
CGA 188245	62	19	—	—	2	14	—	49
CGA 217495	9	—	4	—	59	—	—	—
CGA 91304	2	12	29	—	—	15	—	—
CGA 118244	—	—	4	—	4	—	—	—
CGA 217496	—	—	—	—	—	—	—	—
Fraction 7	—	30	—	—	—	14	—	—
Fraction 8	—	40	—	—	—	57	—	18
Fraction 9	—	—	—	—	6	—	—	—
Fraction 10	—	—	53	—	—	—	—	2
Fraction 11	—	—	9	—	—	—	—	9
Fraction 12	—	—	2	1.5	—	—	—	—
Fraction 13	—	—	—	—	—	—	37	—
Fraction 14	—	—	—	—	—	—	32	—
Fraction 15	—	—	—	—	—	—	32	—
Fraction 16	—	—	—	34	—	—	—	—
Fraction 17	—	—	—	42	—	—	—	—
Fraction 18	—	—	—	4	—	—	—	—
Fraction 19	—	—	—	8	—	—	—	—
Fraction 20	—	—	—	2	—	—	—	—
Fraction 21	—	—	—	23	—	—	—	3
Fraction 22	—	—	—	3	—	—	—	—
Fraction 23	—	—	—	—	—	—	—	14
Fraction 24	—	—	—	—	—	—	—	4
Faeces								
Propiconazole	—	7	15	6	—	14	14	18
CGA 188245	10	8	6	—	9	—	—	11
CGA 91305	7	8	8	—	5	8	—	8

From Cresswell & Hopkins (1989)
—, not identified; IV, intravenous
[a] This dose was administered after 14 unlabelled doses
CGA 188245, (propyl) γ-hydroxy-propiconazole
CGA 217495, dioxalane 4-carboxy acid derivative of propiconazole
CGA 91304, keto-de-dioxolanated propiconazole
CGA 118244, (propyl) β-hydroxy-propiconazole
CGA 217496, dioxalane 4-methylcarboxy acid derivative of propiconazole
CGA 91305, hydroxy-de-dioxolanated propiconazole

males (29%) show a significantly higher rate of cleavage of the dioxolane ring than was found by Bissig (1986). Indications were that oxidation of the propyl side-chain was a significant reaction. Samples from pretreated animals had a different pattern to untreated animals, indcating induction of metabolism (Cresswell & Hopkins, 1989).

The pattern of results found by Cresswell & Hopkins (1989), with two or three components predominating in each group, differs from that found by Mücke (1983), where no component was present at >10%.

Samples from bile-duct cannulated male rats (Bissig, 1992; see above) were investigated for metabolites by TLC. Sixteen metabolite fractions were detected in the urine, the primary urine metabolite (U11; 5.5% of the administered dose) was identified as an α-hydroxy acid derivative of propiconazole. The primary radioactive component of faeces was unchanged propiconazole. There were approximately 16 metabolites/fractions found in samples of bile; the main biliary metabolites were not identified, but the neutral and non-polar components had retention factors (R_f) that were similar to those of some urinary metabolites (Bissig, 1992).

Hens

Samples from hens given 10 mg of [U-^{14}C]phenyl propiconazole for 8 days (Doweyko, 1990a; see above) were investigated by TLC, HPLC and MS after sub-sampling, solvent extraction and, in the case of kidney, incubation with glucuronidase and arylsulfatase. Three major metabolites (propiconazole, CGA118244 and CGA91305) represented >80% of the residue in most tissues, with the metabolite pattern differing between tissues. The metabolite profile in the kidney altered after incubation with arylsulfatase but not with glucuronidase. These results demonstrate that cleavage of the dioxolane ring occurs in hens, with retention of the cleavage products (Doweyko, 1990a).

Goats

Samples of urine, milk and liver from one lactating goat receiving 10 daily doses of 5 mg of triazole-labelled propiconazole (Fisher & Cassidy, 1980; see above) were extracted, fractionated and analysed by two-dimensional TLC, electrophoresis and ion-exchange chromatography either before or after Kjeldahl/sulfuric acid reactions; twelve analytical standards were available for comparison. Conjugation was investigated by incubation with glucuronidase or arylsulfatase. Most metabolites were not conclusively identified. The majority of metabolites in the urine (92%), but <20% of those in the milk and liver were considered to contain both triazole and chlorophenyl rings. TLC patterns for milk and liver were qualitatively similar (Madrid & Cassidy, 1981).

TLC comparison with urine samples from rats (Mücke, 1979) showed that the major urinary metabolites in rats and goats differ, but that the general pattern and extent of metabolism is similar. The primary reaction in both species was oxidation of the propyl side-chain of the dioxolane ring to carboxylic acids. Sulfate conjugation was more extensive in rats than in goats (Madrid & Cassidy, 1981).

Milk and tissue samples from goats given four daily doses of 125 mg of [U-^{14}C]phenyl propiconazole in gelatin capsules were analysed by LSC then extracted and investigated for metabolites by MS, TLC and HPLC (including comparison with eight analytical standards). Most radioactivity was extracted into acetonitrile. Three main metabolites (propiconazole,

Table 7. Levels of metabolites in tissues and milk from goats given four daily doses of 125 mg of [U-^{14}C]phenyl propiconazole

Sample	Concentration (ppm) or % of sample radioactivity	Metabolite						
		Propiconazole	CGA118244	CGA93105	D	E	F	G
Liver	(ppm)	0.63	0.95	0.72	0.67	ND	ND	ND
	(%)	14	21	16	15	ND	ND	ND
Kidney	(ppm)	0.12	0.25	0.47	0.83	ND	ND	ND
	(%)	5	9	18	31	ND	ND	ND
Tenderloin	(ppm)	<0.01	0.01	0.02	0.015	ND	ND	ND
	(%)	1.7	13	30	19	ND	ND	ND
Omental	(ppm)	0.01	0.02	0.02	ND	ND	ND	ND
fat	(%)	20	34	31	ND	ND	ND	ND
Milk	(ppm)	ND	0.05	0.05	0.01	0.01	0.01	0.02
	(%)	ND	23	24	6	5	6	11

From Doweyko (1990b)
ND, not detected

Table 8. Acute toxicity of propiconazole

Species	Strain	Sex	Route	Vehicle	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Purity (%)	Reference
Rat	Tif:RAIf	Male and female	Oral	CMC	1517	—	93	Bathe (1978)
Mouse	Tif:MAG	Male and female	Oral	CMC	1490	—	93	Bathe (1979a)
Rat	Tif:RAIf	Male and female	Dermal	None	>4000	—	93	Bathe (1979b)
Rabbit	NZW	Male and female	Dermal	None	>6000	—	93	Ullmann (1979a)
Rat	Tif:RAIf	Male and female	Inhalation (4-h, nose only, MMAD approximately 2.6 µm)	Ethanol	—	>5	91	Hartmann & Gfeller (1988)

CMC, carboxymethylcellulose; NZW, New Zealand White; MMAD, mass median aerodynamic diameter

CGA 118244 and CGA 91305) represented a significant proportion of the radioactivity in a particular tissue (Table 7), with an unidentified compound representing >30% of the radioactivity in kidney samples. Milk samples were unaltered by treatment with glucuronidase, but hydrolysis by arylsulfatase indicated the presence of sulfate conjugates. Tissue samples were not investigated for the presence of conjugates (Doweyko, 1990b).

2. Toxicological studies

2.1 Acute toxicity

(a) General toxicity

Most of the studies of acute toxicity with propiconazole were performed before the adoption of test guidelines and GLP, but the overall quality of these studies was adequate to determine the acute toxicity of propiconazole. The results are summarized in Table 8. Propiconazole is of moderate acute toxicity via the oral route and low acute toxicity via the dermal and inhalation routes. There were no specific signs of toxicity reported.

(b) Ocular irritation, dermal irritation and dermal sensitization

Propiconazole was moderately irritating to rabbit skin (Ullmann, 1978a) and produced only minimal irritation to rabbit eyes (Ullmann, 1978b). Weak reactions were seen in 3 out of 19 guinea-pigs in an "optimization" test for skin sensitization (Ullmann, 1979b). A pos-

itive result was reported in a test for skin sensitization in guinea-pigs that was performed to a protocol for the Magnusson & Kligman maximization test (Sommer, 1999).

2.2 Short-term studies of toxicity

Oral toxicity was investigated in 4-week and 90-day studies in rats; 13- and 17-week studies in mice; and 90-day and 1-year studies in dogs. Dermal toxicity has been investigated in a 21-day (15 exposures) study in rabbits. A 13-week (65 exposures) inhalation study was performed in rats.

(a) Oral administration

Mice

Groups of 40 male CD1 (ICR) BR mice received diets containing propiconazole (purity, 92%; batch, FL850083) at a concentration of 0, 20, 500, 850, 1450 or 2500 ppm for up to 13 weeks. At 4 weeks and 8 weeks, 10 mice per group were sacrificed, with the remainder being sacrificed at 13 weeks. Routine examinations included survival, clinical signs, body weight and food consumption. Ophthalmoscopy was performed before dosing and before termination. Blood for clinical chemistry investigations (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, sorbitol dehydrogenase and cholesterol) was taken before sacrifice. An extensive necropsy examination was performed, but only brain and liver weights were taken. Only livers were examined histopathologically, including staining with oil red O. Statements of compliance with GLP were provided, but the study does not comply with OECD guideline 408 owing to the limited range of examinations undertaken.

Homogeneity and achieved content of the diet were acceptable. Achieved intakes were 0, 2.8, 71, 121, 199 or 360 mg/kg bw per day. There were no deaths during the study, nor any clinical signs associated with exposure to propiconazole. Body-weight gain was reduced at 2500 ppm from the first week of dosing (0.8 g versus 2.2 g in the controls). Similar clinical chemistry results were seen at 4, 8 and 13 weeks (Table 9), with decreases in

Table 9. Findings in male mice given diets containing propiconazole for up to 13 weeks

	Dietary concentration (ppm)					
	0	20	500	850	1450	2500
4 weeks (n = 10):						
Liver weight (g)	1.3 ± 0.1	1.2 ± 0.2	1.5 ± 0.1	1.8 ± 0.2*	2.5 ± 0.3	2.8 ± 0.4
ALT (U/l)	24 ± 8	26 ± 8	29 ± 17	42 ± 17	56 ± 24*	86 ± 28*
SDH (U/l)	26 ± 5	30 ± 6	39 ± 22	45 ± 11*	58 ± 18*	86 ± 28*
Cholesterol (U/l)	129 ± 26	121 ± 32	122 ± 27	92 ± 18*	81 ± 22*	47 ± 18*
13 weeks (n = 20):						
Liver weight (g)	1.4 ± 0.1	1.4 ± 0.1	1.6 ± 0.2*	1.9 ± 0.2*	2.4 ± 0.4*	2.9 ± 0.3*
ALT (U/l)	22 ± 9	23 ± 6	25 ± 9	35 ± 17	53 ± 15*	79 ± 30*
SDH (U/l)	22 ± 4	25 ± 5	25 ± 5	31 ± 11*	45 ± 10*	58 ± 21*
Cholesterol	122 ± 22	113 ± 24	102 ± 22*	86 ± 24*	75 ± 20*	67 ± 19*
All animals combined (n = 40):						
Hepatocyte hypertrophy	0	0	10*	35*	40*	40*
Hepatocyte necrosis	1	0	4	9*	31*	34*
Hepatocyte vacuolation	0	1	2	5*	15*	33*

From Potrepka & Turnier (1991a)
ALT, alanine aminotransferase; AST, aspartate aminotransferase; SDH, sorbitol dehydrogenase
* $p < 0.05$

cholesterol at 500 ppm and greater and increases in sorbitol dehydrogenase and alanine aminotransferase activities at 850 ppm and greater. Gross histopathology findings identified a number of changes to the livers of animals receiving diets containing propiconazole at 850 ppm and greater; these findings were confirmed histopathologically. Liver weights were increased by >10% at dietary concentrations of 500 ppm and greater by week 4 (Table 9). Hepatocyte necrosis and vacuolation were significantly increased at 850 ppm and greater, with hypertrophy increased at dietary concentrations of 500 ppm and greater (Table 9). An increase in hepatocellular necrosis at 500 ppm is not statistically significant. Histopathological findings did not increase notably with duration of dosing (Potrepka & Turnier, 1991a).

The NOAEL was 500 ppm (equal to 71 mg/kg bw per day) on the basis of statistically significant increases in liver weight, hepatocyte necrosis and serum activities of alanine aminotransferase and sorbitol dehydrogenase at 850 ppm (equal to 121 mg/kg bw per day).

Groups of 20 male and female CD1 (ICR) BR mice received diets containing propiconazole (purity, 92%; batch, FL850083) at a concentration of 0, 20, 500, 850, 1450 or 2500 ppm for males and 0, 20, 500 or 2500 ppm for females, for 17 weeks. Routine examinations included survival, clinical signs, body weight and food consumption. Ophthalmoscopy was performed before dosing and before termination. Blood for clinical chemistry investigations (aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and cholesterol) was taken at 13 weeks (non-fasted) and 17 weeks (fasted). An extensive necropsy examination was performed, but only brain and liver weights were taken. Only livers were examined histopathologically, including specific staining for fat vacuolation. Statements of compliance with GLP were provided, but the study does not comply with OECD guideline 408 owing to the limited range of examinations undertaken.

Homogeneity and achieved content of the diet were acceptable. Achieved intakes were 0, 2.7, 65, 112, 194 or 352 mg/kg bw per day in males and 0, 3.4, 85 or 434 mg/kg bw per day in females. Two males died during the study (one at 20 ppm and one at 850 ppm); these deaths were not considered to be treatment-related. There were no clinical signs associated with exposure to propiconazole. Body-weight gain was reduced by approximately 10% in both sexes at 2500 ppm; in males this was associated with increased food consumption. Similar clinical chemistry results were seen at both 13 and 17 weeks: serum cholesterol was decreased in males receiving propiconazole at dietary concentrations of 850 ppm and greater; alanine aminotransferase activities were increased in females at 2500 ppm and males at 1450 ppm and greater. Gross histopathology findings identified enlarged livers with discoloured foci in males at 1450 ppm and in both groups at the highest dose. Liver weights were increased dose relatedly by >10% in males at dietary concentrations of 500 ppm and greater, and in females at 2500 ppm (Table 10). Histopathological changes in the liver were seen at 850 ppm and greater in males and in females at the highest dose (Table 10). Increased vacuolation observed at 500 ppm was not reproduced at 850 or 1450 ppm and is not considered to be a biologically significant finding (Potrepka & Turnier, 1991b).

The NOAEL was 500 ppm (equal to 65 mg/kg bw per day) on the basis of changes in clinical chemistry, increases in liver weight and histopathology findings in males at 850 ppm and in females at 2500 ppm.

The findings at 500 ppm in these two studies did not show a consistent pattern and support the choice of this dose as the overall NOAEL.

Table 10. Findings in mice given diets containing propiconazole for 17 weeks

Parameter	Dietary concentration (ppm)					
	0	20	500	850	1450	2500
Males						
Liver weight (g)	1.4 ± 0.1	1.4 ± 0.2	1.7 ± 0.2*	1.8 ± 0.2*	2.5 ± 0.3	2.8 ± 0.4
Hepatocyte hypertrophy	0	0	4	14*	20*	20*
Hepatocyte necrosis	1	0	2	4	10*	18*
Vacuolation	0	0	6*	2	3	16*
ALT (U/l)	17 ± 3	33 ± 61	28 ± 24	29 ± 8	65 ± 23*	128 ± 70*
Cholesterol (U/l)	119 ± 20	104 ± 24	105 ± 24	91 ± 25*	66 ± 33*	67 ± 29*
Females						
Liver weight (g)	1.2 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	—	—	2.1 ± 0.3*
Hepatocyte hypertrophy	0	0	0	—	—	17*
Hepatocyte necrosis	0	0	0	—	—	6*
Hepatocyte vacuolation	0	0	0	—	—	3
ALT (U/l)	17 ± 4	19 ± 7	21 ± 11	—	—	61 ± 25*
Cholesterol (U/l)	83 ± 20	93 ± 69	76 ± 22	—	—	64 ± 25
AST	45 ± 9	47 ± 15	55 ± 18	—	—	68 ± 21*

From Potrepka & Turnier (1991b)
ALT, alanine aminotransferase; AST, aspartate aminotransferase
*$p < 0.05$

Rats

Groups of 10 male and 10 female RAIf rats were given propiconazole (purity, 91.9%; batch, P4-6) at a dose of 0, 50, 150 or 450 mg/kg bw per day via gavage in 2% carboxymethyl cellulose for 28 days. Animals were observed routinely for mortality, clinical signs, body weight and food consumption. Haematology examinations were performed on samples obtained from five animals of each sex per group before sacrifice; investigations of clinical chemistry (cholesterol not measured) and urine analysis were performed on samples from the remaining five animals of each sex per group. Ophthalmoscopic examinations and investigations of auditory response were performed before dosing and before termination. The study was performed before GLP and did not comply with the main requirements of OECD guideline 407 (1981) as only the liver was examined histopathologically.

Three females died during the study—two from the group at the highest dose and one from the group at the intermediate dose; one of the animals at the highest dose was in a poor condition before death and this death might have been treatment-related. Clinical signs (sedation, dyspnoea and ruffled coat) related to administration of propiconazole were seen in females at a dose of 450 mg/kg bw per day during the first week of the study only. Body-weight gain was reduced by approximately 20% in males at the highest dose; female body weights were similar in all groups. Food consumption was significantly reduced in females at the highest dose and there was a pattern of reduced food consumption in males at the highest dose. Reductions in erythrocyte volume fraction, haemoglobin and erythrocyte numbers were seen in females at the highest dose (Table 11). Increased concentrations of serum glucose were seen in both sexes at 450 mg/kg bw per day (Table 11) and reductions in concentrations of serum chloride were detected in females at the highest dose (Table 11). Variations in plasma proteins were within the physiological ranges and not considered to be adverse. Activities of serum enzymes of liver damage were similar in all groups. Liver weights were increased in all groups of females and males receiving propiconazole at a dose of 150 mg/kg bw per day or greater (Table 11), but were considered to be adaptive and not adverse. The results of urine analysis did not indicate any reduced renal performance. The increased liver weights at 150 mg/kg bw per day and greater were consistent with liver

Table 11. Findings in rats given propiconazole by gavage for 28 days

Parameter	Sex	Dose (mg/kg bw per day)			
		0	50	150	450
Haematology (n = 5)					
Erythrocytes (10^6/µl)	Male	7.3	7.6	7.6	7.6
	Female	7.3	7.3	7.0	6.6*
Erythrocyte volume fraction (%)	Male	41	43*	44	42
	Female	42	43	42	39*
Haemoglobin (mmol/l)	Male	8.4	8.9	8.9	8.7
	Female	8.6	8.7	8.7	7.7*
Clinical chemistry (n = 5)					
Glucose (mmol/l)	Male	6.9	6.5	7.1	7.4
	Female	6.2	6.9	6.8	8.2*
Chloride (mmol/l)	Male	98.5	97.5	97.8	97.0
	Female	101.3	100.4	100.9	96.8*
Liver (n = 10)					
Liver weight (g)	Male	13.8 ± 1.7	13.5 ± 2.3	18.2 ± 1.7*	18.9 ± 3.1*
	Female	8.6 ± 1.1	11.6 ± 0.4*	13.9 ± 0.9*	14.3 ± 0.8*
Liver hypertrophy	Male	0	0	4	10*
	Female	0	0	8*	10*
Liver necrosis	Male	0	0	1	0
	Female	0	0	0	3

From Basler & Gfeller (1980)
* $p < 0.05$

hypertrophy seen in the histopathological examination (Table 11). Three females from the group at 450 mg/kg bw per day had necrosis of the liver parenchyma (Basler & Gfeller, 1980).

The NOAEL was 150 mg/kg bw per day on the basis of clinical signs of toxicity, liver necrosis and reduced erythrocyte parameters in females at 450 mg/kg bw per day.

Groups of 20 male and 20 femlae Tif:RAIf rats received diets containing propiconazole (purity, 90%; batch, INA 35/1) at a concentration of 0, 240, 1200 or 6000 ppm for 13 weeks. Animals were observed routinely for mortality, clinical signs, body weight and food consumption. Haematology examinations were performed on samples obtained from 10 animals of each sex per group during weeks 4, 8 and 13; investigations of clinical chemistry (cholesterol not measured) and urine analysis were performed on samples from the remaining five animals of each sex per group. Ophthalmoscopic examinations were performed before dosing and before termination. At termination, all animals received a gross examination and a wide range of tissues were examined microscopically after normal and special staining. The study was performed before GLP, but complied with the main requirements of OECD guideline 408 (1981).

Analyses of diet samples confirmed acceptable levels of incorporation. Achieved intakes were 0, 16, 76 or 461 mg/kg bw per day in males and 0, 17, 78 or 481 mg/kg bw per day in females. Body-weight gain was reduced in groups receiving the highest dose from week 2 onwards and to a lesser extent (<10%) in females at 1200 ppm from week 9 onwards. Food consumption in all treated groups was lower than that in the control groups; in groups at the highest dose, the reduction was statistically significant for the first 2 months, but food consumption was higher than that of controls over the final 2 weeks. Food conversion efficiency was reduced at the highest dose only. Changes in erythrocyte parameters in groups at the highest dose were first noted at week 4 and persisted until the end of the study (Table

Table 12. Findings in rats given propiconazole by gavage for 13 weeks

Parameter	Sex	Dietary concentration (ppm)			
		0	240	1200	6000
Body weight (n = 20)					
Body weight (g) at week 13	Male	470 ± 42	463 ± 36	459 ± 46	372 ± 34*
	Female	293 ± 14	286 ± 22	269 ± 22*	235 ± 21*
Haematology (n = 10)					
Erythrocytes (10^6/µl)	Male	8.1	8.1	8.2	7.8*
	Female	7.7	7.9	7.7	7.2*
Erythrocyte volume fraction (%)	Male	44	43	45	43
	Female	44	44	44	41*
Haemoglobin (mmol/l)	Male	9.5	9.5	9.7	9.3
	Female	9.3	9.5	9.4	8.9*
Reticulocytes (%)	Male	5	4	4*	5
	Female	3	4*	4*	4*
Clinical chemistry (n = 10)					
Glucose (mmol/l)	Male	7.5	7.7	8.1*	7.8
	Female	6.4	6.3	7.4*	7.6*
Chloride (mmol/l)	Male	100.0	99.7	99.0	96.8*
	Female	99.7	99.3	98.2	97.4*
γ-GT (U/l)	Male	2.4	2.3	3.0	3.5*
	Female	2.0	2.4	2.4	4.7*
Liver (n = 20)					
Relative liver weight (%)	Male	3.6	3.5	3.7	4.3*
	Female	3.7	3.7	3.9	4.5*

From Sacchsse et al. (1979)
γ-GT, γ-glutamyl transferase
*$p < 0.05$

12). An apparent increase in reticulocytes in all groups of females appears to be associated with a low value for the controls (typical range, 4–5%) and is not considered to be biologically significant. A wide range of statistically significant clinical chemistry changes were seen in animals at the highest dose, including alterations in electrolytes, and increases in blood urea nitrogen, serum protein concentrations and albumin : globulin ratio. Increased serum glucose concentrations were seen in both sexes receiving propiconazole at a dietary concentration of 1200 ppm and greater; in males there was no clear dose–response relationship (Table 12) and the values were within physiological ranges. Reductions in serum concentrations of chloride were detected in the groups at the highest dose (Table 12). With the exception of γ-GT (γ-glutamyl transferase) (Table 12) activities of serum enzymes of liver damage showed no consistent pattern. The results of urine analysis did not indicate any reduced renal performance. Liver weights relative to body weight were increased by approximately 20% in animals receiving propiconazole at a dietary concentration of 6000 ppm (Table 12). Increases in relative brain, heart and adrenal weights appear secondary to reduced body weight. The only histological finding was an increased incidence and severity of splenic haemosiderosis in females at the highest dose (Sacchsse et al., 1979a).

The NOAEL was 1200 ppm (equal to 76 mg/kg bw per day) on the basis of reduced body-weight gain, increased liver weight and altered erythrocyte and clinical chemistry parameters at 6000 ppm.

Dogs

Groups of four male and four female beagle dogs were given diets containing propiconazole (purity, 93%; batch No. IN 35/5) at aconcentration of 0, 50, 250 or 1250 ppm

(equal to 0, 1.3, 6.9 or 35 mg/kg bw per day in males, and 0, 1.6, 7.6 or 36 mg/kg bw per day in females) for 13 weeks. Adequate homogeneity, stability and content of the diets were demonstrated. Dogs were observed routinely for mortality, signs of ill health, hearing ability, body weight and food consumption. Urine analysis, haematology and clinical chemistry investigations were conducted on all dogs during pre-test, and after 4, 8, and 13 weeks of treatment. Ophthalmology examinations were performed on all dogs during pre-test and after 13 weeks of treatment. At termination, all dogs were necropsied, major organs were weighed and an extensive range of tissues, from all animals, was examined histologically. The study complied with OECD test guideline 452 of 1981 and a statement of GLP compliance was provided.

There were no deaths or clinical signs of toxicity. Body-weight gain exhibited some reduction at the intermediate and highest dose in females, but this was considered to be related to the higher body weight at the start of the study (9.9 kg at 1250 ppm versus 7.8 kg in the controls). Food consumption was reduced by approximately 5% in females at the highest dose, but this was possibly a consequence of the reduced energy requirement of these larger animals. There were no adverse effects recorded during, haematology, clinical chemistry or urine analysis investigations. One female at the highest dose had grey streaks on the optic disc of the tapetum. Decreased plasma concentrations of glucose in females were present before dosing and was not considered to be treatment-related. Organ weights were similar in all groups of females when corrected for body-weight differences, but in males at the highest dose, absolute and relative weights of the liver were increased by approximately 10%. Three males at the highest dose and one female at the intermediate dose were reported to have "an increased amount" of lymphoid follicles in the mucous membrane of the pyloric area of the stomach. Possibly related alterations of the gastrointestinal tract were reported in the 1-year study in dogs (see below) and they are thus considered to be treatment-related. The gastrointestinal effects were likely to be cuased by a local reaction to propiconazole, which is irritating to the skin, and the relevance to exposures in humans could not be discounted. The NOAEL for local effects was 250 ppm (equal to 6.9 mg/kg bw per day) on the basis of lymphoid changes in the stomach at 1250 ppm. The NOAEL for systemic effects was 1250 ppm (equal to 35 mg/kg bw per day, the highest dose tested (Sachsse et al., 1980b).

Four groups of five male and five female beagle dogs (and groups of two males and two females for the recovery segment of the study) were given diets containing propiconazole (purity, 90.2%; batch No., FL831527) for 1 year. A recovery subgroup comprising two males and two females in the control group and in the group receiving the highest dose was maintained on untreated diet for 1 month after the end of the 1-year treatment phase. Dietary concentrations were 0, 5, 50 or 250 ppm (equal to 0, 0.17, 1.9 or 8.4 mg/kg bw per day in males, and 0, 0.19, 1.9 or 8.9 mg/kg bw per day in females). A nominal ration of 400 g of food was provided, but this was increased to 500 g for male dogs from week 18 onwards. Adequate homogeneity, stability and content of the diets were demonstrated. Dogs were observed routinely for mortality, signs of ill health, and measurement of body weight and food consumption. Urine analysis, haematology and clinical chemistry investigations were conducted on all dogs during pre-test, and after 3, 6, and 12 months of treatment. Ophthalmology examinations were performed on all dogs during pre-test and after 1 year of treatment. At termination (at 12 months for main groups or 13 months for recovery groups), all dogs were necropsied, major organs were weighed and an extensive range of tissues, from all animals, was examined histologically. The study complied with OECD test guideline 452 of 1981 and a statement of GLP compliance was provided.

Table 13. Incidence of gastrointestinal findings in dogs receiving propiconazole for 1 year

	Dietary concentration (ppm)			
	0 (control)	5	50	250
Males (n = *5*)				
Colon hyperaemia	0	0	0	1
Ileum hyperaemia	0	0	0	1
Jejunum hyperaemia	0	0	0	1
Caecum hyperaemia	0	0	0	1
Duodenum hyperaemia	0	0	1	2
Stomach hyperaemia	0	0	1	3
Females (n = *5*)				
Duodenum hyperaemia	0	2	0	2

From Johnson et al. (1985)

There were no deaths or clinical signs of toxicity. Body-weight gain exhibited transient effects with no clear pattern, but, in an attempt to resolve this, the food ration for males was increased. Food consumption was similar in all groups. There were no adverse effects recorded during ophthalmoscopy, or investigations of haematology, clinical chemistry or urine analysis. Occasional findings, e.g. increased plasma concentrations of glucose at 6 months in females showed a high degree of variability and were not reproduced at other time-points. Organ weights were similar across all groups. Gross pathological examination identified reddening of the gastrointestinal tract, which was confirmed as hyperaemia by microscopic examination (Table 13). The findings were seen predominantly in males in the group receiving the highest dose and were not evident after the recovery phase. The study report gave no indication of the extent or severity of the hyperaemia. Similar results were reported in the shorter-term study in dogs (see above), but there was no evidence of similar effects in the long-term studies in mice and rats. The gastrointestinal effects were likely to be caused by a local reaction to propiconazole, which is irritating to the skin, and the relevance to exposures in humans could not be discounted. The NOAEL for local effects was 50 ppm (equal to 1.9 mg/kg bw per day) on the basis of hyperaemia of the gastrointestinal tract at 250 ppm. The NOAEL for systemic effects was 250 ppm, equal to 8.4 mg/kg bw per day, the highest dose tested (Johnson et al., 1985)

(b) Dermal exposure

Rabbits

Groups of 10 male and 10 female KA 46 New Zealand White rabbits received undiluted propiconazole (purity, 91.9%; batch, P4-6) at a dose of 0, 200, 1000 or 5000 mg/kg bw per day applied to the shorn skin for 6 h per day, 5 days per week, for 3 weeks (total of 15 applications). Five rabbits per group had the application site abraded, for the remaining rabbits, the application site was intact. Routine observations were performed for survival, clinical signs, body weight and food consumption; blood samples for haematology and clinical chemistry were taken before dosing and at termination. Gross and microscopic examinations were performed. This study complies with the main requirements of OECD test guideline 410, but was performed before GLP.

There were no deaths. Local skin lesions, which might have lead to enhanced absoption, were present at intact and abraded sites in all treated groups at much increased incidence and severity relative to controls. Clinical signs (tremor, dyspnoea, ataxia) were seen

from day 4 onwards at 1000 mg/kg bw per day and greater. Body-weight gain was reduced in males at the highest dose. In females, the impact on body weight was compounded by the wide intergroup variation in initial weights, but an approximate 300 g reduction in body weight at 5000 mg/kg bw per day, between days −3 and +1, is considered to be treatment-related. A range of clinical chemistry changes were seen, but most were without any clear pattern. Findings likely to be related to treatment included: increases in blood urea nitrogen concentration, bilirubin and urea in males at the highest dose; erythrocyte count and haemoglobin concentration were decreased by approximately 10% in females at the highest dose, γ-GT activity was increased in both sexes at 5000 mg/kg bw per day, and serum concentration of sodium, which normally shows minimal variation, was increased in males, but decreased in females at the highest dose. Liver weights were increased by approximately 25% in the groups receiving the highest dose. There were no gross or microscopic changes indicative of systemic toxicity (Sachsse et al., 1980a).

This study failed to determine a NOAEL for local effects. The NOAEL for systemic effects was 200 mg/kg bw per day (5 days per week) on the basis of clinical signs at 1000 mg/kg bw per day.

(c) Inhalation exposure

Rats

Groups of 20 male and 20 female RAIf rats were exposed to aerosols of propiconazole (purity, 91.9%; batch, P4-6) as an 80% solution in acetone, for 6 h per day, 5 days per week, for 13 weeks. Concentrations in the atmosphere were determined gravimetrically as 21, 85 or 191 mg/m^3 (0.021, 0.085, 0.191 mg/l) and the particle size was determined to be in the respirable range (80% of particles had a diameter of <7 μm); an air control group and an acetone control group were also used. Animals were observed routinely for deaths, clinical signs, body weight and food consumption. Samples of blood for haematology and clinical chemistry (fasted samples) examinations was taken from 10 rats of each sex per group in weeks 6 and 13 (within a 1-h period). Ophthalmoscopy examinations were performed pre-test and before sacrifice. Gross and microscopic examinations were performed. The study complied with the main elements of OECD guideline 413, but was performed before GLP.

Two deaths (one male in the control group and one female at the lowest dose) were not considered to be treatment-related. There were no clinical signs of toxicity. Body-weight gain was reduced consistently (15–20%, $p < 0.01$) in females at the highest dose; reductions in body-weight gains in females at the lowest dose and in males at the intermediate dose were not part of a dose–response relationship and were not considered to be a direct effect of propiconazole. There were no consistent changes in haematology parameters. Changes in a number of clinical chemistry parameters were seen, but only reductions in serum concentrations of glucose in all groups of females exhibited any degree of consistency. However, the values were within the normal physiological range and are not considered to be adverse. Liver weight was increased by approximately 10% in females exposed at 191 mg/m^3 (0.191 mg/l). There were no ophthalmic, gross or histopathological changes associated with exposure to propiconazole (Sachsse et al., 1979b).

The no-observed-adverse-effect concentration (NOAEC) was 85 mg/m^3 (0.85 mg/l; approximately 20 mg/kg bw per day) on the basis of reduced body weights in females at 191 mg/m^3 (0.191 mg/l).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 52 male and 52 female CD1 mice received diets containing propiconazole (purity, 92%; batch, P4-6) at a concentration of 0, 100, 500 or 2500 ppm for 104 weeks. Satellite groups of 12 male and 12 female mice were fed diets containing propiconazole at the same dietary concentrations and sacrificed after 53 weeks of treatment. Routine observations included clinical signs, mortality, body weight, food consumption, food efficiency and water consumption. Haematology, clinical chemistry and urine analysis were performed at 1 year and before sacrifice at 102–104 weeks. A gross examination was performed, a range of organs were weighed and a comprehensive histopathological examination performed. Results were analysed using a range of appropriate statistical tests.

Mean intakes of propiconazole were 10, 49 or 344 mg/kg bw per day in males and 11, 56 or 340 mg/kg bw per day in females at 100, 500 or 2500 ppm respectively. There were no abnormal clinical signs or increases in palpable masses associated with propiconazole. During the first 26 weeks, mortality was significantly increased in males at 2500 ppm. Overall survival was adequate for a study of carcinogenicity in mice: >50% at 92 weeks in all groups except males at the highest dose, for which survival dropped below 50% after week 84. At 2500 ppm, body-weight gain was reduced significantly throughout the study in male and female mice. Body-weight gain was reduced by approximately 10% in males at 100 and 500 ppm during weeks 1–13, but as this was not seen subsequently, nor in females, it was not considered to be biologically relevant. Food consumption was increased in males at the highest dose during the entire study and during the first 5 months in females. Erythrocyte volume fraction and haemoglobin concentration were decreased at 2500 ppm in males at 1 and 2 years and in females at 2 years. Aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase activity were significantly increased in males at the highest dose by approximately twofold at week 52 and approximately fourfold at week 104, and to a lesser extent in females at the highest dose at week 52. Cholesterol concentrations were reduced in a statistically significant manner in males at the highest dose at week 52 and in females at the highest dose at termination. Slight, non-statistically significant effects on alanine aminotransferase and cholesterol were present at 500 ppm in males. No evidence of renal toxicity was evident in the results of urine analysis.

Liver weight was significantly increased in males and females at the highest dose and in males at the intermediate dose (Table 14). The gross examination revealed a higher incidence of liver masses and/or enlarged livers in males and females receiving propiconazole at a dietary concentration of 2500 ppm. The gross findings were confirmed by microscopic examination. In the group receiving the highest dose at 12 months, there was an increase in liver weight, hepatocellular necrosis and inflammatory cell infiltration (Table 14). After 24 months at 2500 ppm, there was an increase in hepatocellular enlargement and vacuolation and eosinic foci in males and females and pigmented Kupffer cells in males (Table 14). At 500 ppm, there was an increase in hepatocyte vacuolation in females, with increases in hepatocellular enlargement and eosinophilic foci in males (Table 14). An increase in the incidence of benign and malignant liver cell tumours was seen in males at 2500 ppm in both interim and main study groups. In an addendum to the original report (Hardisty, 1991), liver findings were re-examined according to contemporary criteria. The overall conclusions were unchanged after the re-evaluation and the results are presented in Table 14. There were no treatment-related effects on organs other than the liver (Hunter et al., 1982a).

Table 14. Histopathology results in mice given diets containing propiconazole for up to 104 weeks

	Dietary concentration (ppm)			
	0	100	500	2500
Interim group, 53 weeks:				
Males				
No. of animals examined	11	11	11	9
Liver weight (g)	2.3 ± 0.4	2.5 ± 0.4	2.8 ± 0.6*	4.4 ± 1.4*
Hepatocyte enlargement, mild/moderate	2	2	5	9*
Hepatocyte necrosis	0	0	0	4
Inflammatory cell infiltration	1	0	2	6
Hepatocellular adenoma only (No. of mice)	1	0	4	1
Hepatocellular carcinoma (No. of mice)	0	0	0	3
Females				
No. of animals examined	12	11	11	12
Liver weight (g)	1.7 ± 0.4	1.7 ± 0.2	1.7 ± 0.3	2.4 ± 0.5
Hepatocyte enlargement	0	0	0	7
Main group, 102 to 104 weeks:				
Males				
No. of animals examined	53	53	51	55
Liver weight (g)	3.2 ± 1.8	2.9 ± 1.2	3.4 ± 1.1	7.4 ± 2.8*
Hepatocyte enlargement, mild/moderate	8	3	16*	44*
Hepatocyte necrosis	5	2	4	3
Inflammatory cell infiltration	30	26	26	38
Basophilic foci	5	5	7	1
Eosinophilic foci	1	1	5*	6*
Pigmented Kupffer cells	7	8	8	37*
Hepatocyte vacuolation	7	5	7	19*
Hepatocellular adenoma only (No. of mice)	11	7	9	22*
Hepatocellular carcinoma (No. of mice)	16	9	13	22
Hepatocellualr adenoma & carcinoma combined	27	16	22	44**
Females				
No. of animals examined	52	53	53	52
Liver weight (g)	2.0 ± 1.0	1.9 ± 0.5	1.9 ± 0.3	3.0 ± 1.3*
Hepatocyte enlargement, mild/moderate	0	0	0	24*
Hepatocyte necrosis	3	5	2	2
Inflammatory cell infiltration	30	26	17	21
Basophilic foci	0	2	2	0
Eosinophilic foci	1	1	0	4
Hepatocyte vacuolation, mild/moderate	3	2	7	16*
Hepatocellular adenoma only (No. of mice)	5	0	2	6
Hepatocellular carcinoma (No. of mice)	1	1	0	3

From Hunter et al. (1982a)
* $p < 0.05$
** $p = 0.0013$; Fisher exact test

The NOAEL for non-neoplastic effects was 100 ppm, equal to 10 mg/kg bw per day, on the basis of hepatocellular lesions at 500 ppm. The no-observed-effect level (NOEL) for neoplasia was 500 ppm (equal to 49 mg/kg bw per day) on the basis of a significant (p = 0.0013) increase in the combined incidence of liver tumours at 2500 ppm in males.

Groups of 80 Crl:CD1 (ICR) BR male mice were given diets containing propiconazole (purity, 92.4%) at a concentration of 0, 100, 500 or 850 ppm for 18 months. Fifty animals per group were used for evaluation of oncogenic potential. An additional 10 animals per group were designated for blood chemistry investigations, scheduled at weeks—1, 9, 14, 53 and 79. Ten animals per group were used for interim sacrifice at weeks 9 and 53. Animals were observed routinely for mortality, clinical signs, body weight and food con-

sumption. Clinical chemistry investigations for cholesterol concentration, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and sorbitol dehydrogenase activities were performed on 10 males per group. Extensive gross examinations were performed and a wide range of tissues was preserved from all animals. Only samples of liver were examined histologically, but the examinations included an independent peer review. Results were analysed with a range of appropriate statistical tests. Statements of compliance with GLP were provided, but the study did not comply fully with OECD test guideline 451 owing to the limited histopathological examinations undertaken and poor survival.

Homogeneity and achieved intakes were satisfactory. The mean intakes of propiconazole were 11.0, 59 and 108 mg/kg bw per day in groups receiving dietary concentrations of 100, 500 and 850 ppm, respectively. Survival in the main group was relatively poor (30–40% at week 78), but was unaffected by propiconazole. No clinical signs nor behavioural changes indicative of a treatment-related effect were observed. Body-weight gain and food consumption were increased at 850 ppm during the first month, but were subsequently lower than those of the controls; body-weight gain was 15% lower than that of controls at 1 year. Slightly reduced body-weight gain (up to 10%) was evident at 500 ppm. Treatment-related decreases in plasma cholesterol concentrations were recorded throughout the study in animals receiving dietary concentrations of 500 (10–35%) and 850 ppm (20–40%); the decreases at weeks 9 and 14 were statistically significant in the group at 850 ppm. Sorbitol dehydrogenase activities were increased (by approximately 50%) in mice treated at 850 ppm at weeks 9 and 14. Mean liver weights were increased at 500 and 850 ppm (Table 15). Weights of other organs (brain, heart, kidney and testes) were not notably altered by consumption of propiconazole.

Treatment-related macroscopic findings of enlarged livers and more liver nodules/masses were seen at week 78 (Table 15). Evidence of liver changes was seen during histopathological examinations at all time-points. At week 9, fatty change was slightly increased at 100 ppm and greater, and hypertrophy was present in animals at 500 ppm and greater. Hypertrophy was the only clearly treatment-related finding at 500 ppm at 53 and 79 weeks. Animals at the highest dose had a broad pattern of hepatic effects, including hepatocellular adenomas (Table 15). The incidence of adenomas at 500 ppm (3 out of 50) is at the lower end of the control range of 6–18% reported in CD1 mice in the same test facility (Schaetti, 1999) and is not considered to be biologically significant; the incidence at 850 ppm was above that for historical and concurrent controls and was considered to be treatment-related (Gerspach, 1997).

The NOAEL for non-neoplastic effects was 100 ppm (equal to 11 mg/kg bw per day) on the basis of reduced cholesterol, reduced body-weight gain (10%) and increased liver weights/hypertrophy at 500 ppm. The fatty change noted at 9 weeks in 2 out of 10 animals at 100 ppm did not progress and was not considered to be biologically significant.

The NOAEL for tumours was 500 ppm (equal to 59 mg/kg bw per day) on the basis of a significant increase in the incidence of liver adenomas at 850 ppm.

In a follow-up investigation, preserved liver sections from animals in the control group and at the highest dose that were sacrificed at 9 weeks in the study by Gerspach (1997) were stained for proliferating cell nuclear antigen (PCNA). The results showed no difference between animals in the control group (labelling index, 0.05 ± 0.04%) and animals treated with propiconazole (0.06 ± 0.04%) (Weber, 1997).

Table 15. Liver findings in male mice given diets containing propiconazole for up to 79 weeks

Finding	Dietary concentration (ppm)			
	0	100	500	850
Liver weight (g)				
Week 9	2.25	2.12	2.50 (+11%)	2.98* (+32%)
Week 53	2.58	2.55	2.93 (+14%)	2.86* (+11%)
Week 79	2.58	2.58	2.93 (+14%)	3.07* (+19%)
Gross findings in week 79				
Enlarged liver	12/60	9/60	12/60	21/60
Liver mass	1/60	3/60	3/60	4/60
Liver nodule	0/60	0/60	0/60	1/60
Microscopic findings				
Week 9:				
Livers examined	10	10	10	10
Fatty change	0	2	2	9*
Hepatocellular hypertrophy	0	0	6*	10*
Necrosis	2	3	3	5
Necrosis monocellular	0	0	1	3
Lymphohistiocytic infiltration	2	2	1	6
Week 53:				
Livers examined	10	10	10	10
Hepatocellular hypertrophy	1	3	6*	8*
Necrosis	5	3	6	6
Hyperplasia, Kupffer cells	0	1	1	2
Amyloidosis	5	5	8	9
Week 79:				
Livers examined	50	50	50	50
Hepatocellular hypertrophy	15	18	28*	29*
Kupffer cell pigmentation	3	5	3	11*
Focus of cellular change	0	0	1	6*
Fatty change	21	14	15	13
Inflammatory cell infiltration	4	2	2	6
Hemangioma	0	0	1	1
Malignant lymphoma, systemic infiltration	0	1	1	1
Hepatocellular adenoma	1	0	3	10*
Hepatocellular carcinoma	1	3	2	2
Total hepatocellular tumours	2	3	5	12*

From Gerspach (1997)
*$p < 0.05$

Rats

Groups of CD Sprague-Dawley rats (50 of each sex per group for tumorigenic evaluation; 10 of each sex per group for interim sacrifice at 52 weeks; 10 of each sex per group for haematology; and 10 of each sex per group for blood chemistry and urine analysis) were fed diets containing propiconazole (purity, 91.9%; batch P4-60) at a concentration of 100, 500 or 2500 ppm for 107–109 weeks. Routine observations included clinical signs, mortality, body weight, food and water consumption. Haematology and urine analysis, clinical chemistry, ophthalmoscopy and hearing tests were performed at approximately 6, 12, 18 and 24 months; with an additional clinical chemistry analysis at 33 weeks. Extensive gross and microscopic examinations were performed (including special stains). Results were assessed using a range of appropriate statistical tests. The study complied with OECD guideline 453 and statements of compliance with GLP were provided.

Results of diet analyses are not reported, but the procedure used for preparation of diet was shown to be acceptable in other studies. Achieved intakes were 0, 3.6, 18 or 96 mg/kg bw per day in males and 0, 4.6, 23 or 131 mg/kg bw per day in females. Survival of the rats in groups treated with propiconazole, especially at the highest dose, was higher

Table 16. Findings in Sprague-Dawley rats given diets containing propiconazole for up to 2 years

	Dietary concentration (ppm)							
	0		100		500		2500	
	Males	Females	Males	Females	Males	Females	Males	Females
Body-weight gain (g)								
Weeks 0–26	419	189	418	188	409	175*	365*	139*
Weeks 26–52	113	81	118	75	108	78	83*	37*
Weeks 52–104	105	105	120	115	110	116	70*	79
Body weight (g)								
Week 78	794	495	806	496	802	482	701*	361*
Week 104	820	506	840	525	797	512	716*	395*
Food consumption: body-weight gain weeks 0–26[a] Food intake (% controls)	10.6	16.8	10.6	16.6	10.7	17.7	11.9	22.0
Weeks 1–26	100	100	99	99	99	98	98	95
Weeks 27–52	100	100	99	97	98	97	96	92
Haemoglobin (g %)								
Week 26	15.3	14.7	15.6	14.7	15.3	15.2	15.0	14.5
Week 52	16.1	16.1	16.7*	16.7*	16.3	16.3	16.0	16.0
Week 78	14.3	13.2	14.3	14.9*	13.9	14.8*	13.0*	13.6
Week 103	13.1	14.6 ± 0.4	14.5	14.2 ± 1.2	14.3	13.7 ± 1.2	14.0	12.8* ± 1.7
Erythrocyte count (10^6/mm^3)								
Week 26	8.8	7.6	8.9	7.6	8.5	8.0*	8.7	7.8
Week 52	7.5	6.3	7.6	6.2	7.6	6.3	7.8	6.5
Week 78	8.0	6.4	8.0	6.8	7.9	6.5	8.0	6.5
Week 103	6.2	7.0 ± 0.3	7.7*	6.8 ± 0.8	7.4*	6.6 ± 0.5	7.6*	6.5 ± 0.7
Chloride (mmol/l)								
Week 26	105	105	103*	101*	100*	100*	99*	103*
Week 52	103	101	101*	101	101	100*	101*	102
Week 78	101	100	101	100	101	99	102	98
Glucose (mg %)								
Week 26	—	130 ± 15	—	128 ± 15	—	108 ± 14*	—	109 ± 10*
Week 33	—	129 ± 12	—	137 ± 12	—	119 ± 15	—	120 ± 18
Week 52	—	111 ± 12	—	108 ± 14	—	102 ± 10	—	98 ± 16*
Week 78	—	131 ± 18	—	123 ± 13	—	114 ± 12*	—	111 ± 9*
Week 104	—	122 ± 15	—	120 ± 8	—	109 ± 19	—	114 ± 8
Albumin : globulin ratio								
Week 26	1.2	1.7	1.3*	1.4*	1.2	1.3*	1.3*	1.2*
Week 52	1.3	1.6	1.2	1.5	1.3	1.4*	1.3	1.3*
Week 78	1.2	1.4	1.1	1.3*	1.2	1.1*	1.2	1.1*
Exocrine pancreas atrophy Liver	—	1/59	—	3/61	—	6/62[#]	—	9*/65
No. of animals examined	64	67	67	69	66	67	65	67
Enlarged hepatocytes	2	1	0	2	2	2	5	13*
Benign liver tumours	2	1	1	1	2	0	2	2
Cholangioma	0	0	0	0	0	0	0	1
Dilatation of uterine lumen	—	4/58	—	10/63	—	9/63	—	17/65*

From Hunter et al. (1982b; 1985)
[a] The only time period calculated
[#] $p = 0.057$
* $p < 0.05$

than that in the control group, possibly because of lower body weight and decreased food consumption in treated animals. Overall survival was >50% in all groups of males at termination and in all groups of females treated with propiconazole. Body-weight gain and food consumption were decreased in females at the highest dose throughout the study and in males at the highest dose during the first year. At 500 ppm, growth and food efficiency was decreased slightly in females during the first 26 weeks (Table 16), but not subsequently and was not considered to be adverse. Urine analysis, ophthalmoscopic and hearing inves-

tigations did not identify any adverse effects. At 2500 ppm, numerous changes in haematology (erythrocyte parameters) and clinical chemistry were observed; some were also present at 500 and 100 ppm, although dose–response relationships were often absent, the direction of changes was reversed between the sexes (Table 16) and values were within normal ranges. At interim kill and termination, increased liver weight (by approximately 20%) was observed in males and females at 2500 ppm. At 52 weeks, relative weights of the heart and ovaries was increased in females at 2500 ppm.

Histopathological examination identified the liver as the primary target organ, with increases in the incidence of enlarged liver cells seen at 2500 ppm, which was statistically significant in females. Increased atrophy of the exocrine pancreas was evident in females at a dietary concentration of 500 ppm and greater. The overall incidence of tumours was increased in males, with no clear dose–response relationship or clear increases in specific tumours, this finding was possibly related to the increased survival in treated animals. Liver cell tumours were slightly higher in animals at the highest dose (Table 16), but were within normal background ranges and when combined with the increased survival are not considered to indicate a tumourigenic response to propiconazole. Reticulum cell tumours of the pancreas were present in three animals in the satellite groups, but were not seen in the main group and are thus not considered to indicate a carcinogenic response to propiconazole (Hunter et al., 1982b; 1985).

The NOAEL for tumours was 2500 ppm (96 mg/kg bw per day) the highest dose tested. Propiconazole is not carcinogenic to rats.

The NOAEL for non-neoplastic effects was 500 ppm (equal to 18 mg/kg bw per day) on the basis of increased luminal dilatation of the uterus in females and liver lesions and reduced body-weight gain in both sexes at 2500 ppm.

Dogs

No long-term studies in dogs were submitted

2.4 Genotoxicity

Propiconazole has been investigated in vitro for its ability to induce gene mutations in bacteria and mammalian cells, chromosomal aberrations in mammalian cells, and unscheduled DNA synthesis in mammalian cells. Propiconazole has also been tested in vivo in two studies of micronucleus induction and a study of dominant lethal mutation (Table 17). The highest concentrations used were justified on the basis of results of screening for toxicity/cytotoxicity. All studies gave negative results and complied with the OECD guidelines extant at the time of performance. The only study performed to GLP was the study of micronucleus induction in mice. Although the studies do not meet current OECD guidelines, the overall extent of the genotoxicity database is considered adequate.

A number of additional studies giving negative results were reviewed by the JMPR in 1987 (Annex 1, reference 52).

The Meeting concluded that propiconazole was unlikely to be genotoxic.

Table 17. Results of studies of genotoxicity with propiconazole

End-point	Test object	Concentration or dose	Purity (%) [Batch]	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA98, 100, 1535, 1537, 1538	20–5120 µg/plate ± S9; in DMSO	90.7 [103119]	Negative +S9 Negative –S9	Deparade & Arni (1983)
Gene mutation	Mouse lymphoma L5178Y $Tk^{+/-}$ cells	7.8–125 µg/ml ± S9; in DMSO	90.7 [103119]	Negative +S9 Negative –S9	Strasser & Muller (1982a)
Cell transformation	BALB 3T3 mouse embryo cells	1.2–18.5 µg/ml; in DMSO	90.7 [103119]	Negative	Strasser & Muller (1982b)
Chromosomal aberration	Human peripheral lymphocytes (one donor)	1–180 µg/ml ± S9; in DMSO	89.7 [P301064]	Negative +S9 Negative –S9	Strasser & Arni (1984)
Unscheduled DNA synthesis	Tif:RAIf rat hepatocytes	0.7–83 ng/ml; in DMSO	90.7 [103119]	Negative	Puri & Muller (1982)
In vivo					
Micronucleus formation	Bone marrow from Chinese hamsters (eight of each sex per group)	0, 307, 615, or 1230 mg/kg bw by gavage; in arachis oil	90.7 [103119]	Negative	Strasser & Arni (1987)
Micronucleus formation	Bone marrow from Ico:CD1 mice (five of each sex per group)	0, 80, 1600, or 3200 mg/kg bw by gavage; in arachis oil	92.4 [OP303011]	Negative	Deparade (1999)
Dominant lethal mutation	Tif:MAGf (SPF) mice (20 males)	0, 165, or 495 mg/kg bw by gavage; in methylcellulose)	90.7 [103119]	Negative	Hool & Müller (1979) Caresa (1988)

S9, 9000 × *g* supernatant from mouse liver cells induced with Aroclor 1254 or with propiconazole

2.5 Reproductive toxicity

(a) Multigeneration study

In a two-generation study (two litters per generation), groups of CD rats received diets containing propiconazole (purity, 89.7%; batch FL830377) at a concentration of 0, 100, 500 or 2500 ppm during pre-mating, gestation, lactation and weaning. Groups consisted of 15 males and 30 females. Dosing was initiated 12 weeks before mating for F_{1a} and F_{2a} litters. Animals were observed for mortality, clinical signs, body weight, food consumption, mating performance and reproductive outcome. All P_1 parental animals and 10 weanlings of each sex per group received a gross examination, brain and reproductive organs were weighed, and liver and reproductive organs were examined histopathologically. The study complied with OECD guideline 416 (1983) and statements of compliance with GLP were provided.

Dietary analyses showed satisfactory levels of incorporation, and mean intakes of propiconazole during the study were approximately 10, 50 or 250 mg/kg bw per day. There were no signs of toxicity in P_0 or P_1 males other than a slight (approximately 10%) reduction in body-weight gain at 2500 ppm. Females at the highest dose exhibited reduced body-weight gain (approximately 20%) and reduced food consumption during most stages of the study. Females at the intermediate dose showed reductions in body-weight gain (approximately 10%; $p < 0.01$). Mating performance, fertility and duration of gestation were similar in all groups, although the degree of variation in values for controls hindered comparisons between matings. Lower weights of pups at day 21 were often associated with larger litter sizes in the F_{1a} and F_{1b} matings, but total litter weights were lower at 2500 ppm (Table 18). In the second generation, both pup weight and litter size were reduced at 2500 ppm, and in the F_{2b} litters, pup weights were also reduced ($p < 0.01$) at 500 ppm. The main effect on F_2 litter size was poor survival during lactation. Organ weights such as brain and testes/epididymides showed no consistent pattern between matings, with reductions in absolute values or increases in relative values being secondary to body-weight changes. There were no

Table 18. Litter findings (means) in a multigeneration study in rats given diets containing propiconazole

Finding	Dietary concentration (ppm)			
	0	100	500	2500
F_{1a} day 21				
Pup weight (g)	47	43	46	35*
No. of pups	6.8	7.2	7.4	7.4
Litter weight (g)	320	310	340	260
F_{1b} day 21				
Pup weight (g)	42	44	45	34**
No. of pups	6.3	7.7	7.7	7.2
Litter weight (g)	264	339	346	245
F_{2a} day 21				
Pup weight (g)	42	42	41	31**
No. of pups	7.8	7.1	7.0	6.2**
Litter weight (g)	328	298	287*	192
Viable F2a pups				
Day 0	12.5	13.0	10.8	9.2**
Day 4	7.9	7.8	7.5	6.7**
Day 14	7.8	7.2	7.0	6.2**
F_{2b} day 21				
Pup weight (g)	47	47	43**	35**
No. of pups	7.7	8.0	7.8	5.7**
Litter weight (g)	362	376	335	200
Viable F_{2b} pups				
Day 0	12.8	13.4	12.7	11.3
Day 4	7.8	8.0	7.8	7.3
Day 14	7.7	8.0	7.8	5.7*

From Borders et al. (1985)
*$p < 0.05$
**$p < 0.01$

treatment-related gross pathology findings. Histopathological examination of the reproductive organs found no notable effects of treatment. Treatment-related changes (hepatocellular swelling and clear-cell changes) were found in the livers of both parental and weanling animals at 2500 ppm and in parents from groups at 500 ppm.

The NOAEL for parental toxicity was 100 ppm (equivalent to 7 mg/kg bw per day) on the basis of reduced body-weight gain in dams and hepatotoxicity at 500 ppm.

The NOAEL for reproductive outcome was 500 ppm (equivalent to 35 mg/kg bw per day) on the basis of reduced pup survival at 2500 ppm in the F_2 generation.

The NOAEL for offspring toxicity was 100 ppm (equivalent to 7 mg/kg bw per day) on the basis of reduced pup body weights at day 21 in the F_{2b} litters at 500 ppm (Borders et al., 1985).

(b) Developmental toxicity

Rats

Groups of 25 mated female Tif:RaIf rats were given propiconazole (purity, 91.9%; batch P4-6) at a dose of 0, 30, 100 or 300 mg/kg bw per day by gavage in 2% carboxymethyl cellulose on days 6–15 of gestation. One third of the fetuses were evaluated by Wilson sectioning, the rest were stained for skeletal investigation. Three dams in the group receiving

Table 19. Findings in rats given propiconazole by gavage during organogenesis

Parameter	Dose (mg/kg bw per day)							
	0		30		90		360/300	
Body-weight gain (g)								
Days 6–8	7 ± 4		4 ± 5		3 ± 5*		3 ± 5*	
Days 6–16	47 ± 8		44 ± 10		46 ± 7		40 ± 11*	
Food consumption (g)								
Days 0–6	19		21		21		20	
Days 6–7	21		22		19		19	
Days 7–8	21		21		20		17*	
Days 8–9	22		22		20*		19*	
Days 9–10	22		23		21		17*	
Maternal body weight (g)								
Day 6	285		283		292		285	
Day 8	292		287		295		288	
Day 16	331		328		333		326	
Day 20[a]	313		303		315		308	
Litter parameters								
Live fetuses per litter	12.3		13.5		13.7		13.0	
Fetal weight (g)	3.5		3.4		3.4		3.4	
% male fetuses	52		49		48		46	
Fetal findings (fetuses/litters)								
Cleft palate	0	0/22	0	0/21	1	1/22	2	2/22
Cleft/hare lip	0	0/22	0	0/21	2	2/22	0	0/22
Renal papilla short	32/141	16/22	27/148	9/20	40/156	18/22	57/148*	19/22
Renal papilla missing	4	4	4	3	8	3	16*	12
Dilated ureter	38	16	21	8	38	16	63*	20
Rudimentary ribs	0/129	0/22	1/136	1/21	4/146*	4/22*	53/137**	16/22**
Sternebrae not ossified	49		54		83*		99**	

Marcsisin et al. (1987)
[a] Minus uterine contents
* $p < 0.05$
** $p < 0.01$

the highest dose died. No malformations were recorded at 300 mg/kg bw per day, but two fetuses at the intermediate dose had malformations. No cleft palates were recorded in the study. Delayed ossification, particularly of the phalangeal nuclei and calcaneum was seen at the highest dose. The level of detail in the report was insufficient to permit a NOAEL to be derived (Fritz, 1979).

Groups of 24 mated female Crl:COBS CD(SD)BR VAF/Plus rats were given propiconazole (purity, 92.1%; batch FL850083) at a dose of 0, 30, 90 or 360/300 mg/kg bw per day by gavage in aqueous 3% corn starch + 0.5% Tween® on days 6–15 of gestation. The highest dose was reduced from 360 to 300 mg/kg bw per day after three to five doses owing to maternal toxicity. Dams were sacrificed on day 20 of gestation and fetuses were examined. Approximately half of the fetuses in each litter were examined for visceral abnormalities, the remainder were examined for skeletal abnormalities after staining with alizarin red. This study complied with OECD guideline 414 (1981) and statements of compliance with GLP were provided.

One control female died due to complications associated with an early birth. Severe compound-related maternal toxicity (lethargy, ataxia, salivation) was observed at the highest dose during the first five days of dosing at 360 mg/kg bw per day. Dams from the group at the highest dose also exhibited reduced body-weight gain (Table 19) at 360 mg/kg bw per

day. The reduced body-weight gain persisted after the dose was reduced to 300 mg/kg bw per day. Reduced body-weight gain was also noted at the intermediate dose during the first days of dosing, but terminal body weights were similar to those of controls. There were no effects on litter sizes, fetal viability, litter size, pup weight or sex ratio. One fetus at the intermediate dose had multiple malformations (cleft lip and palate, micromelia and club foot), which were not considered to be clearly related to treatment. At the highest dose, one fetus had multiple malformations (anasarca, cleft palate, hydromelia and protruding tongue), another fetus at the highest dose had cleft palate. Cleft palate is very rare, but not unknown, in CD rats; incidence in controls ranged from 0% to 0.3%. Evidence of delayed development of the urinary system was seen at the highest and intermediate doses, together with an increase in rudimentary ribs and unossified sternebrae (Table 19). It is uncertain if these findings were secondary to maternal toxicity as fetal weights were similar in all groups.

The NOAEL for maternal toxicity was 90 mg/kg bw per day on the basis of severe signs of toxicity at 300 mg/kg bw per day. Although the maternal body-weight gains were significantly decreased at 90 mg/kg bw per day during days 6–8 of gestation only, this effect was considered to be temporary and secondary to reduced food consumption.

The NOAEL for developmental toxicity was 30 mg/kg bw per day on the basis of reduced ossification and the presence of rudimentary ribs at 90 mg/kg bw per day (Marcsisin et al., 1987).

In a study to investigate the findings of cleft palate reported by Marcsisin et al. (1987), mated female Crl:COBS CD(SD)BR VAF/Plus rats (178 controls; 189 test) were given propiconazole (purity, 92.1%; batch FL850083) at a dose of 0 or 300 mg/kg bw per day by gavage in aqueous 3% corn starch + 0.5% Tween® on days 6–15 of gestation. Dams were sacrificed on day 20 of gestation and the uterine contents were examined. Fetal examinations focused primarily on the palate. Statements of compliance with GLP were provided.

Three animals from the group treated with propiconazole were found dead, and another was sacrificed owing to problems associated with early delivery. Clinical signs

Table 20. Findings in rats given propiconazole by gavage during organogenesis

Parameter	Dose (mg/kg bw per day)			
	0		300	
Body-weight gain (g)				
Days 6–8	9.3 ± 6.1		–2.7 ± 8.8*	
Days 6–16	61 ± 13		41 ± 13*	
Days 16–20	58 ± 13		63 ± 16*	
Litter parameters				
Live fetuses per litter	13.7 ± 3.2		13.1 ± 3.4*	
Fetal weight (g)	3.5		3.3*	
% male fetuses	49		50	
Fetal findings (fetuses/litters)				
Cleft palate	0/2122	0/155	2/2064	2/158

From Mallows (1987)
* $p < 0.05$
** $p < 0.01$

including ataxia, coma, lethargy, abnormal breathing and ptosis were present in animals treated with propiconazole. Food consumption and body-weight gain were reduced during dosing with propiconazole, but partially recovered on cessation of dosing (Table 20). There were no unusual findings at the necropsy examination. Fetal weight and litter size were reduced in the group receiving propiconazole (Table 20). Two fetuses (from different litters) with cleft palate were seen out of 2064 fetuses from dams treated with propiconazole (0.1%), versus zero out of 2122 fetuses in the control group.

This study was not designed to determine a NOAEL.

Cleft palate is a very rare but occasional finding in rats in control groups and there are published data (Khera, 1985) that indicate that testing compounds at maternally toxic doses is associated in some way with the induction of a number of malformations, including cleft palate. The low incidences seen in these two studies cannot be discounted as being related to treatment with propiconazole, but it is considered that they are unlikely to be seen in the absence of maternal toxicity.

Rabbits

Groups of 19 inseminated, female New Zealand White rabbits were given propiconazole (purity, 92.1%; batch FL850083) at a dose of 0, 100, 250 or 400 mg/kg bw per day by gavage in aqueous 3% corn starch + 0.5% Tween® on days 7–19 of gestation. Dams were observed for clinical signs, mortality, body weight and food consumption, and sacrificed on Day 29 of gestation; fetuses were examined after caesarean section. All fetuses were examined for visceral abnormalities by dissection and for skeletal abnormalities after staining with alizarin red. Owing to processing errors, a large number of fetuses (20–40%) were damaged (disarticulated) before skeletal examinations; the damaged fetuses were examined by experienced technicians for evidence of skeletal abnormalities. Overall, the number of fetuses examined in this study is considered to be acceptable. This study complied with OECD guideline 414 (1981) and statements of compliance with GLP were provided.

Two does (one at the lowest dose and one at the intermediate dose) were found dead. One doe in the control group, one at the intermediate dose and five at the highest dose were sacrificed having aborted or delivered early. Maternal toxicity was evident at 400 mg/kg bw per day (reduced food consumption, body-weight loss; abnormal stools; abortion/early delivery) and to a lesser extent at 250 mg/kg bw per day (reduced feed consumption and body-weight loss) (Table 21). Slight, but consistent, reductions in food consumption and body-weight gain were evident at 100 mg/kg bw per day, but were not associated with any adverse effects on fetuses. Food consumption and body-weight gain in groups receiving propiconazole were greater than those of controls after cessation of dosing (Table 21). There were no necropsy findings indicating treatment-related effects. One animal at the highest dose resorbed an entire litter. In does with viable fetuses, litter sizes and fetal weights were similar in all groups (Table 21). One fetus at the intermediate dose had a number of abnormalities. The only treatment-related finding was an increased incidence of fully formed thirteenth ribs at 400 mg/kg bw per day (Table 21). There was also an increase in the number of resorptions at 400 mg/kg bw per day that was associated with resorption of an entire litter by one dam.

The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of body-weight loss at 250 mg/kg bw per day; the slight effects on food consumption and body-weight gain at 100 mg/kg bw per day were not considered to be adverse.

Table 21. Summary of findings of a study of developmental toxicity in rabbits given propiconazole by gavage

Finding	Dose (mg/kg bw per day)			
	0	100	250	400
Food consumption (g)				
Days 7–8	183 ± 24	162 ± 21	136 ± 32*	79 ± 31*
Days 14–15	164 ± 48	121 ± 63	95 ± 60*	69 ± 45*
Day 26–27	90 ± 45	107 ± 37	104 ± 45	151 ± 48*
Body-weight gain (g)				
Days 7–10	18 ± 3	5 ± 3	–47 ± 5*	–111 ± 8*
Day 20–24	46 ± 8	94 ± 4	118 ± 7*	159 ± 6*
Pregnant	15	18	17	18
Litter parameters				
Viable litters	14	17	15	12*
Litter size	7.2	8.6	8.7	7.2
Resorptions	0.7	0.7	0.7	2.1*
Fetus weight (g); male/female	43.0/44.2	44.4/43.1	42.8/41.1	42.8/43.2
% live males/litter	55	49	50	41
Fetal findings—fetal incidence (%)				
Fully formed thirteenth rib	35/101 (35)	63/146 (43)	58/130 (41)	63/93* (68)

From Raab et al. (1986)
* $p < 0.05$

The NOAEL for developmental toxicity was 250 mg/kg bw per day on the basis of increases in fully formed thirteenth ribs and abortions at 400 mg/kg bw per day. The NOAEL for teratogenicity was 400 mg/kg bw per day, the highest dose tested (Raab et al., 1986).

2.6 Special studies

(a) Neurotoxicity

(i) Acute oral neurotoxicity in rats

No studies of acute neurotoxicity were submitted; however, no evidence of neurotoxicity was apparent in any of the available studies.

(ii) Short-term study of oral neurotoxicity

No short-term studies of neurotoxicity were submitted; however, no evidence of neurotoxicity was apparent in any of the available studies.

(b) Assay for initiation/promotion of liver foci

After finding liver tumours in mice, a study was performed to investigate the effect of propiconazole (purity, 89.7%; batch 301064) on focal proliferative changes in the livers of young rats. Groups of 45 male and 45 female Tif:RAIf rats aged 1 day were given *N*-diethylnitrosamine (DEN) at a dose of 15 mg/kg bw by intraperitoneal administration or 0.5 ml of saline by intraperitoneal administration. After weaning on day 22, animals were given control diet or diets containing phenobarbitone at 500 ppm or propiconazole at 2000 ppm. Subgroups of five animals of each sex were sacrificed after 14, 28 or 56 days of treatment and the livers were examined using periodic acid Schiff, haematoxylin/eosin or histochemical staining for γ-glutamyl transpeptidase-positive foci. Treatment with propiconazole or phenobarbital ± DEN produced significant increases (15–30%) in liver weights. Numbers of γ-glutamyltranspeptidase-positive foci were increased by treatment with phenobarbital or propiconazole, with the increases being more marked after an initi-

Table 22. Number of γ-glutamyl transferase-positive foci in livers from rats treated with propiconazole or phenobarbital

Group	Days of treatment					
	14 days		28 days		56 days	
	Male	Female	Male	Female	Male	Female
Saline + control diet	0	14	1	15	0	38
Saline + 500 ppm phenobarbital	0	0	53	69	11	34
Saline + 2000 ppm propiconazole	0	143	142	165	422	244
N-diethylnitrosamine + control diet	55	179	103	71	121	198
N-diethylnitrosamine + 500 ppm phenobarbital	493	612	367	284	613	660
N-diethylnitrosamine + 2000 ppm propiconazole	552	488	382	516	1178	1189

From Fröhlich et al. (1984)

ating exposure to DEN (Table 22). The variable background rate in females confounds any comparisons of the relative responses in the two sexes. The size of the foci was unaffected by treatment with phenobarbital or propiconazole. The results indicate that propiconazole acts in a similar manner to phenobarbital (Fröhlich et al., 1984).

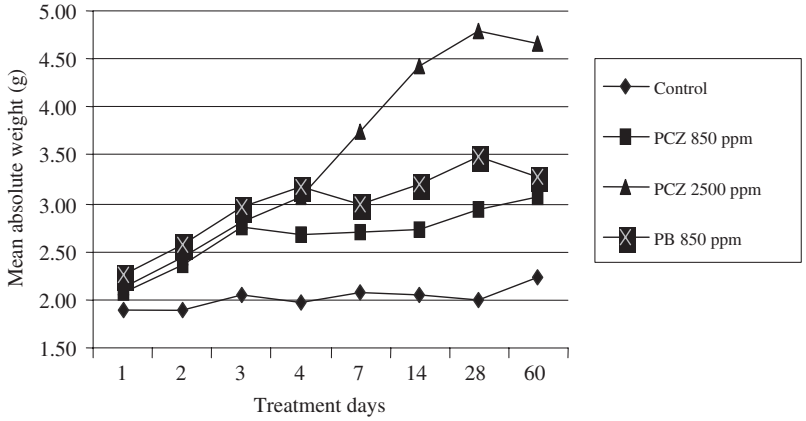
(c) Induction of liver enzymes and cell proliferation

Propiconazole belongs to a class of conazole pesticides, which have complex effects on the hepatic and non-hepatic microsomal monooxygenase systems in vertebrate species. They can act as both inducers and inhibitors of cytochrome P450, depending on the tissue and the specific conazole. A number of studies have been performed to investigate the pattern of microsomal enzyme induction produced by propiconazole.

The pattern of xenobiotic-metabolizing enzymes induced by propiconazole was investigated in a study performed in 1984. Groups of male RAI rats and MAG mice (six in the test groups and eight in the control group) were given propiconazole (purity, 90.7%) at a dose of 0, 20, 80, 160 or 320 mg/kg bw per day by gavage in 2% carboxymethyl cellulose for 2 weeks. After a 24-h fast, the animals were sacrificed and the liver cytosol and microsomal preparations investigated for enzyme activities, protein, nucleic acid and phospholipid contents. Samples from two animals in the control group and two animals at the highest dose were studied by electron microscopy. Animals reciving propiconazole at a dose of 20 mg/kg bw per day showed increases in liver weight. At 80 mg/kg bw per day and above, there were increases in microsomal protein, phospholipid and cytochrome P450 content and activities of ethoxycoumarin deethylase, epoxide hydrolase, UDP-glucuronyltransferase and γ-GT in both rats and mice (Table 23). Cytosolic protein (in mice) and glutathione *S*-transferase (rats and mice) were increased at 160 mg/kg bw per day and above (Table 23). A marked proliferation of smooth endoplasmic reticulum was seen in samples from the animals at 320 mg/kg bw per day (Waechter et al., 1984).

In a study designed to characterize the extent and time-dependency of hepatocyte proliferation and investigate a possible mechanism for the tumorigenic effect observed, groups of five CD1 male mice were given diets containing propiconazole (purity, 92.4%) at a concentration of 0, 850 or 2500 ppm. To examine hepatocyte cell proliferation, each animal received a single intraperitoneal injection of bromodeoxyuridine (BRDU) at a dose of 100 mg/kg bw 2 h before sacrifice. Cell proliferation was assessed by BRDU-immunohistochemistry/image analysis after treatment for 1, 2, 3, 4, 7, 14, 28 or 60 days. Mice treated with phenobarbital (purity, 99%), a known mouse mitogen, at a concentration of 850 ppm were investigated as for those treated with propiconazole.

Figure 1. Mean absolute weight of the liver in mice given diets containing propiconazole or phenobarbital for up to 60 days



From Weber (1999)
PCZ, phenobarbital; PB, phenobarbital

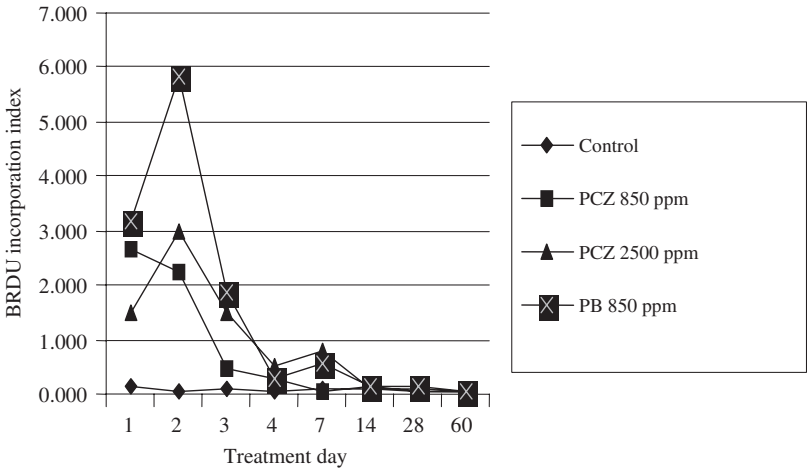
Table 23. Biochemical findings in livers from animals given propiconazole by gavage for 14 days

Finding	Dose (mg/kg bw per day)				
	0	20	80	160	320
Rats					
Liver weight (g)	3.1	3.5*	3.3*	4.1**	4.7**
DNA (mg)	31	NP	NP	NP	36*
Cytosolic protein (mg/g)	66	58	69		
Microsomal protein (mg/g)	9	9	14**	13**	17**
Microsomal phospholipid (mg/g)	6	5	8**	9**	12**
Cytochrome P450 (nmol/g)	12	11	22**	28**	40**
Ethoxycoumarin deethylase (nmol/min per g)	5	4	9**	17**	31**
Epoxide hydrolase (nmol/min per g)	154	132	300**	579**	852**
UDP-glucuronyltransferase (µmol/min per g)	1	1	2**	3**	4**
γ-GT (nmol/min per g)	18	22	24*	17	41**
Glutathione *S*-transferase (µmol/min per g)	87	77*	99	175**	269**
Mice					
Liver weight (g)	4.7	5.2*	5.1*	6.0**	6.8**
DNA (mg)	5	NP	NP	NP	7**
Cytosolic protein (mg/g)	62	59	67	79**	71
Microsomal protein (mg/g)	11	9	12	18**	21**
Microsomal phospholipid (mg/g)	7	7	9	12**	14**
Cytochrome P450 (nmol/g)	16	15	28*	49**	63**
Ethoxycoumarin deethylase (nmol/min per g)	19	24	23	31**	55**
Epoxide hydrolase (nmol/min per g)	63	51	71	160**	212**
UDP-glucuronyltransferase (µmol/min per g)	619	404**	664	890**	1080**
Glutathione *S*-transferase (µmol/min per g)	290	301	380*	421**	422**

From Waechter et al. (1984)
γ-GT, γ-glutamyl transeferase; NP, not performed
*$p < 0.05$
**$p < 0.01$

Dietary incorporation was acceptable, and mean intakes were 127 or 353 mg/kg bw per day for propiconazole and 139 mg/kg bw per day for phenobarbital. No deaths occurred during the study and no treatment-related clinical signs were reported. Body-weight development and food consumption was suppressed during the first days of treatment at 2500 ppm. No effects on body-weight development, food consumption or body weights were noted in groups treated with propiconazole or phenobarbital at 850 ppm. Time- and dose-related increases in absolute and relative weights of the liver were observed in animals treated with propiconazole (Figure 1).

Figure 2. Bromodeoxyuridine incorporation index in mouse hepatocytes after exposure to propiconazole or phenobarbital



From Weber (1999)
PCZ, phenobarbital; PB, phenobarbital

Enlarged and/or speckled livers were found at macroscopic examination in all groups treated with propiconazole or phenobarbital. One hepatic nodule was found in each of the groups treated with propiconazole at a high or low dose. Hepatocellular hypertrophy was seen microscopically in animals treated with propiconazole: the mean severity, increasing with time, ranged from minimal (1 day of treatment) to moderate/marked (28 and 60 days of treatment). Treatment with phenobarbital led to a progression of hepatocellular hypertrophy similar to that observed in the group treated with propiconazole at 2500 ppm. The hypertrophy caused by propiconazole affected all lobular compartments of the liver, but was predominantly centrilobular, while phenobarbital almost exclusively affected centrilobular or midzonal hepatocytes. Hepatocellular necrosis was present in all groups, showing a dose-related increase in incidence and severity in the groups treated with propiconazole. Panlobular hepatocyte vacuolation (minimal) was observed in some animals from all treatment groups at the begining of the study. Centrilobular vacuolation was observed towards the end of the treatment with a high incidence (almost all animals) and severity (minimal to marked) from day 7 to 60 in animals treated with propiconazole at 2500 ppm, but only sporadically in animals treated with propiconazole or phenobarbital at 850 ppm.

Mitotic activity and incorporation of BRDU was increased markedly in most treated animals during days 1–4 of treatment, returning to control values by day 14 (Fig. 2). The increased incorporation of BRDU was primarily in the centrilobular/midzonal hepatocytes. The magnitude of this response was lower with propiconazole at 2500 ppm than with phenobarbital at 850 ppm, contrary to the results for liver weight (Fig. 1), indicating that a short mitogenic response is not the sole mechanism underlying the hepatocellular proliferative effects of propiconazole.

This study showed propiconazole to be a mouse liver mitogen with a degree of similarity to phenobarbital, but of a lower potency in terms of BRDU incorporation (Weber, 1999).

Groups of six male Crl:CD1(ICR)BR mice aged 8 weeks were given diets containing propiconazole (purity, 92.4%; batch No. OP.303011) at a concentration of 0, 850 or 2500 ppm (equal to 0, 149 and 578 mg/kg bw per day) for 14 days. A further group of six

mice was treated with phenobarbital at 850 ppm (equal to 145 mg/kg bw per day). Body weights and food consumption were measured daily and any clinical signs were recorded. After 14 days, the mice were killed and their carcass weights recorded after bleeding, and they were examined macroscopically. The livers of all animals were removed, weighed and stored in liquid nitrogen. The liver samples were separated into microsomal and cytosolic fractions and the following biochemical parameters were investigated: protein content of microsomal and cytosolic fractions; microsomal cytochrome P450; microsomal 7-ethoxyresorufin-*O*-dealkylase activity (EROD); 7-pentoxyresorufin-*O*-dealkylase activity (PROD); microsomal coumarin 7-hydroxylase activity; regio- and stereoselective microsomal hydroxylation of testosterone; microsomal lauric acid 11- and 12-hydroxylation; microsomal UDP-glucuronosyltransferase activity; cystolic glutathione *S*-transferase activity and microsomal epoxide hydrolase activity.

There were no deaths or clinical signs of toxicity. Body weight was similar in all groups. Absolute weights of the liver were increased by 40–100% in all treatment groups (Table 24). A dose-related induction of specific microsomal enzyme activities was seen with a corresponding increase in total protein content (Table 24) in the animals treated with propiconazole. The pattern of enzyme induction produced by propiconazole was similar, but not

Table 24. Induction of microsomal enzymes in male mice given diets containing propiconazole or phenobarbital for 14 days (% of control values)

Parameter	Dietary concentration (ppm)		
	Phenobarbital	Propiconazole	
	850	850	2500
Absolute liver weight	163	140	199
Microsomal cytochrome P450 content	239	300	389
Microsomal pentoxyresorufin *O*-depentylase	3534	3024	5524
Microsomal coumarin 7-hydroxylase	480	534	2384
Microsomal testosterone 2β-hydroxylation	466	298	531
Microsomal testosterone 6α-hydroxylation	779	502	715
Microsomal testosterone 6β-hydroxylation	500	366	524
Microsomal testosterone 15β-hydroxylation	526	316	977
Microsomal testosterone 16β-hydroxylation	5.7[a]	5.2[a]	6.4[a]
Microsomal testosterone oxidation to androstenedione	179	589[b]	652[b]
Microsomal testosterone 2α-hydroxylation	0[a]	2.7[a,b]	2.7[a,b]
Total microsomal testosterone oxidation	356	440	555
Microsomal epoxide hydrolase[d]	179	172	321
Immunoblot goat anti-rat CYP2B1 (Cyp2b) Band 1	2743	2608	3049
Immunoblot goat anti-rat CYP2B1 (Cyp2b) Band 2	581	810	579
Immunoblot goat anti-rat CYP2B1 (Cyp2b) Band 3	351	439	617
Immunoblot mAb p6 (Cyp3a)	577	658	1068
Microsomal ethoxyresorufin *O*-deethylase[c]	232	219	388
Microsomal testosterone 16α-hydroxylation	194	214	262
Microsomal lauric acid 11-hydroxylase	271	267	305
Microsomal lauric acid 12-hydroxylase[d]	163	153	161
Microsomal UDP-glucuronosyltransferase	156	156	139
Cytosolic glutathione *S*-transferase	187	158	184
Microsomal testosterone 7α-hydroxylation	229	175	125
Microsomal protein content	97	109	113
Cytosolic protein content	92	97	94
Immunoblot mAb d15 (Cyp1a)[c]	86	124	89
Immunoblot mAb clo4 (Cyp4a)[d]	129	140	157

From Beilstein (1998)

[a] Absolute values, control value below limit of detection

[b] Notable difference between phenobarbital and propiconazole

[c] Indicative of aromatic hydrocarbon response

[d] Indicative of peroxisomal proliferation

identical, to that produced by phenobarbital. The main differences were in the induction of testosterone oxidation to androstenedione and of testosterone 2α-hydroxylation that were induced by propiconazole but not phenobarbital (Table 24). Within the range of microsomal enzyme parameters measured, the overall response to propiconazole is more consistent with a phenobarbital-type mechanism than peroxisome proliferation or aromatic hydrocarbon mechanism. However, mechanisms other than one similar to that used by phenobarbital cannot be conclusively excluded (Beilstein, 1998).

(d) Studies with metabolites

No studies were submitted. Triazolyl acetic acid and triazolyl alanine are produced from propiconazole by plants, but not by mammals. Triazolyl alanine was reviewed by the JMPR in 1989. Triazolyl acetic acid and triazolyl alanine were considered by the 2004 JMPR during the evaluation of triademefon and triademenol.

3. Observations in humans

Medical surveillance of employees in production, formulation and packaging plants from 1982 to April 2000 revealed four cases (out of a total of 139 individuals) of local skin reactions. All four cases occurred during handling of formulations. It cannot be excluded that formulation ingredients may have contributed to the effects described. The occasional occurrence of local effects during handling of formulations of propiconazole is accordance with other reported cases described in the literature. The consistent finding is of local irritation to the skin or mucosa; as all cases involved the formulated propiconazole product, the reactions could have been associated with constituents other than propiconazole. No cases of serious poisoning with propiconazole or formulations containing propiconazole have been reported to Syngenta or found in the public literature (Jaquet, 1991).

An epicutaneous test with technical-grade propiconazole in 20 human volunteers was conducted in 1991 at the University of Göttingen in Germany. No evidence of sensitization or dermal irritation was reported (Fuchs, 1991).

Comments

After oral administration of radiolabelled propiconazole to rats and mice, the radiolabel is rapidly (C_{max} at 1 h) and extensively (>80% of the administered dose) absorbed and widely distributed, with the highest concentrations being found in the liver and kidney. Excretion of the radiolabel is rapid (80% in 24 h) with significant amounts being found in the urine (39–81%) and the faeces (20–50%), the proportions varying with dose, species and sex. There is a significant degree of biliary excretion and subsequent enterohepatic recirculation. There was no evidence for bioaccumulation with tissue or carcass residues being typically <1% of the administered dose 6 days after dosing. Propiconazole is extensively metabolized and <5% of the dose remains as parent compound; however, many metabolites have not been identified. The primary metabolic steps involve oxidation of the propyl sidechain on the dioxolane ring to give hydroxy or carboxylic acid derivatives. Hydroxylation of the chlorophenyl and triazole rings followed by conjugation with sulfate or glucuronide was also detected. There is evidence for only limited cleavage between the triazole and chlorophenyl rings. The extent of cleavage of the dioxolane ring was significantly different according to species and sex, representing about 60% of urinary radioactivity in male mice, 30% in female mice and 10–30% in male rats. In rats, propiconazole is readily absorbed after dermal application (about 30% within 10 h).

Propiconazole has moderate acute oral toxicity in rats and mice (LD_{50}s, about 1500 mg/kg bw) and low acute dermal (LD_{50}s, >4000 mg/kg bw) and inhalation toxicity (LC_{50}, >5 mg/l of air). Propiconazole is not an eye irritant in rabbits, but is irritating to rabbit skin and is a skin sensitizer in guinea-pigs in the Magnusson & Kligman test.

Decreased body-weight gain was seen in short- and long-term studies of toxicity and studies of developmental and reproductive toxicity, and was often linked with reduced food consumption. In studies of repeated doses, liver was the primary target organ for toxicity attributable to propiconazole. In rats, erythrocyte parameters were reduced and a range of clinical chemistry changes were seen, however, with the exception of reduced chloride and cholesterol concentrations, there was no consistent pattern between sexes and studies, and results were generally within the physiological range.

In two studies, mice given diets containing propiconazole at ≥850 ppm for up to 17 weeks had increases in liver weight, reduced concentrations of serum cholesterol and increased hepatocyte hypertrophy, vacuolation and necrosis. The findings were present after 4 weeks and did not progress with increased duration of dosing. The NOAEL was 500 ppm (equal to 65–85 mg/kg bw per day) in both studies.

Rats given propiconazole at 450 mg/kg bw per day for 28 days by gavage exhibited a range of effects. Males had reductions in body-weight gain, while females had clinical signs of toxicity and reductions in erythrocyte parameters. Both sexes had increased liver weights and hepatocyte hypertrophy, with hepatocyte necrosis also being seen in females. Increases in liver weight with hepatocyte hypertrophy were seen at 150 mg/kg bw per day, but these effects were not considered to be adverse and the NOAEL was this dose. In a 13-week dietary study in rats, reductions in body-weight gain, increased relative liver weight and increased γ-glutamyltranspeptidase activity was seen in both sexes at 6000 ppm. In females, erythrocyte parameters were reduced at this dose. The NOAEL was 1200 ppm (equal to 76 mg/kg bw per day).

Dogs appeared to be sensitive to the local effects of propiconazole as manifested by gastrointestinal tract irritation at ≥8.4 mg/kg bw per day; the NOAELs were 250 ppm (equal to 6.9 mg/kg bw per day) after 90 days and 1.9 mg/kg bw per day after 1 year. No systemic effects were seen in dogs receiving a dose of 8.4 mg/kg bw per day for 1 year or 1250 ppm (equal to 35 mg/kg bw per day) for 90 days, the highest doses tested.

In a 3-week (five applications per week) study of dermal toxicity in rabbits, tremors, dyspnoea and ataxia were increased at ≥1000 mg/kg bw per day. The NOAEL was 200 mg/kg bw per day. In a 13-week (5 days per week; 6 h per day) study in rats treated by inhalation, reduced body-weight gain was seen in females at 0.19 mg/l of air; the NOAEC was 0.085 mg/l of air.

The carcinogenic potential of propiconazole was studied in one study in rats and in two studies in mice. In a 2-year dietary study in male and female mice, and an 18-month dietary study in male mice, the liver was the only target organ. At ≥500 ppm, there were decreases in body-weight gain and serum concentration of cholesterol, and increases in liver weight, hepatocellular hypertrophy and hepatocellular vacuolation. The NOAEL for non-neoplastic effects in both studies was 100 ppm (equal to 11 mg/kg bw per day). Propiconazole was a hepatocarcinogen only in male mice, on the basis of significant increases in the incidence of liver tumours at ≥850 ppm (equal to 108 mg/kg bw per day), with a NOAEL of

500 ppm (equal to 59 mg/kg bw per day). Assays for hepatocyte proliferation (measured by bromodeoxyuridine incorporation) in mice showed qualitative similarities between propiconazole and phenobarbital. The doses that produced increases in tumour incidences (≥850 ppm) also produced cell proliferation, increased liver weight and hepatocyte hypertrophy. Studies of liver enzyme induction in mice showed that propiconazole increased the activity of a number of P450s, particularly Cyp2b, and exhibited similar characteristics to a phenobarbital type inducer of xenobiotic-metabolizing enzymes. The progression from P450 (Cyp2b) induction, initial mitogenic response, hepatocyte hypertrophy and increased liver weight to tumours is consistent with a mode of action similar to that of phenobarbital.

At 2500 ppm (96 mg/kg bw per day) in a 2-year dietary study in rats, there were reductions in body-weight gain in both sexes. Increased incidences of enlarged hepatocytes were present in males and increases in atrophy of the exocrine pancreas and dilatation of the uterine lumen in females. Slight (<10%), transient reductions in body-weight gain, variations in clinical chemistry and haematology parameters that fell within physiological ranges at 500 ppm (equal to 18 mg/kg bw per day) were not considered to be adverse. Propiconazole was not carcinogenic in rats at doses of up to 2500 ppm (equal to 96 mg/kg bw per day). The NOAEL in the 2-year study in rats was 500 ppm (equal to 18 mg/kg bw per day).

Propiconazole gave negative results in an adequate battery of studies of genotoxicity in vitro and in vivo. The Meeting concluded that propiconazole was unlikely to be genotoxic.

On the basis of the above consideration of liver tumours in male mice, the high doses required to induce tumours, the likely mechanism of action, the absence of tumorigenicity in rats and the negative results in studies of genotoxicity, the Meeting concluded that propiconazole was unlikely to pose a carcinogenic risk to humans.

In a two-generation study of reproductive toxicity in rats, reproductive parameters were not affected by treatment with propiconazole. At 500 ppm (equivalent to 35 mg/kg bw per day) dams had reduced body-weight gains ($p < 0.01$) and both sexes exhibited hepatoxicity, thus the NOAEL for parental toxicity was 100 ppm (equivalent to 7 mg/kg bw per day). The NOAEL for offspring toxicity was 100 ppm (equivalent to 7 mg/kg bw per day) on the basis of decreased pup body weights in the F_{2b} litters ($p < 0.01$). The NOAEL for reproductive effects was 500 ppm (equivalent to 35 mg/kg bw per day) on the basis of reduced pup survival at 2500 ppm (equivalent to 175 mg/kg bw per day).

Three studies of developmental toxicity were conducted in rats and one in rabbits. In the first study in rats, at the highest dose of 300 mg/kg bw per day there was evidence of maternal toxicity and retarded development, but no malformations. In the second study, propiconazole caused developmental delay (incomplete ossification of sternebrae and rudimentary cervical ribs) at a dose of 90 mg/kg bw per day, which also produced a slight, transient reduction in food consumption and body-weight gain at the initiation of dosing. The NOAEL was 90 mg/kg bw per day for maternal effects and 30 mg/kg bw per day for developmental effects. A low incidence of cleft palate was observed at 90 mg/kg bw per day (one fetus; 0.3%) and at 360/300 mg/kg bw per day (two fetuses; 0.7%) in the presence of severe maternal toxicity. The maternal toxicity included lethargy, ataxia, salivation and reductions in food consumption and body-weight gain at the start of the dosing period. The cleft palate finding was also seen at a low incidence in rats in an extensive study that specifically

investigated the palate and jaw at a single dose of 300 mg/kg bw per day. Cleft palates were detected in 2 out of 2064 fetuses of treated animals, versus none in the 2122 fetuses of controls, in the presence of severe maternal toxicity. Marked maternal toxicity was observed throughout the treatment period, included reductions in food consumption and body-weight gain, ataxia, coma, lethargy and prostration, and three treatment-related deaths among 189 dams. Cleft palate is a very rare but occasional finding in control rats and there were published data that indicated testing compounds at maternally toxic doses is associated in some way with the induction of a number of malformations, including cleft palate.

Propiconazole was not teratogenic in rabbits. The NOAEL for fetal effects was 250 mg/kg bw per day on the basis of an increased incidence of the formation of thirteenth ribs at 400 mg/kg bw per day in the presence of maternal body-weight loss, signs of toxicity and abortions. The NOAEL for maternal toxicity was 100 mg/kg bw per day on the basis of reduced food consumption and body-weight loss at 250 mg/kg bw per day.

No studies of neurotoxicity with propiconazole were available; however, no evidence of neurotoxicity was apparent in any of the available studies.

Humans exposed to formulated products containing propiconazole have shown local irritant reactions. No evidence of sensitization was seen in an epicutaneous test in 20 volunteers.

The Meeting concluded that the existing database on propiconazole was adequate to characterize the potential hazards to fetuses, infants and children.

Toxicological evaluation

The Meeting established an ADI of 0–0.07 mg/kg bw based on the NOAEL of 7 mg/kg bw per day in a multigeneration study of reproductive toxicity in rats and a 100-fold safety factor. This value covers all other end-points and is supported by NOAELs of 11 mg/kg bw per day in a 24-month study in mice, and 18 mg/kg bw per day in a 2-year study in rats. This ADI is protective against the local effects seen in the gastrointestinal tract in dogs (NOAEL, 1.9 mg/kg bw per day), which were considered to be concentration-dependent and hence would merit a safety factor of 25.

An ARfD of 0.3 mg/kg bw was established based on the NOAEL of 30 mg/kg bw per day in the study of developmental toxicity in rats and a 100-fold safety factor. The NOAEL was identified on the basis of slight increases in rudimentary ribs and unossified sternebrae at 90 mg/kg bw per day, which could not be discounted. This provides an adequate margin over the maternal toxicity and cleft palate seen at 300 mg/kg bw per day. The Meeting noted that the highest dose tested in dogs was 35 mg/kg bw per day and that the proposed ARfD would be protective for any potentially acute effects observed in dogs.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	24-month study of toxicity and carcinogenicity[a,e]	Toxicity	100 ppm, equal to 11 mg/kg bw per day	500 ppm, equal to 59 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 59 mg/kg bw per day	850 ppm, equal to 108 mg/kg bw per day
Rat	2-year study of toxicity and carcinogenicity[a]	Toxicity	500 ppm, equal to 18 mg/kg bw per day	2500 ppm, equal to 96 mg/kg bw per day
		Carcinogenicity	2500 ppm, equal to 96 mg/kg bw per day[c]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	100 ppm, equivalent to 7 mg/kg bw per day	500 ppm, equivalent to 35 mg/kg bw per day
		Offspring toxicity	100 ppm, equivalent to 7 mg/kg bw per day	500 ppm, equivalent to 35 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity,	90 mg/kg bw per day	300 mg/kg bw per day
		Embryo- or fetotoxicity	30 mg/kg bw per day	90 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	100 mg/kg bw per day	250 mg/kg bw per day
		Embryo- or fetotoxicity	250 mg/kg bw per day	400 mg/kg bw per day
Dog	3-month study of toxicity[a]	Systemic effects	1250 ppm, equal to 35 mg/kg bw per day[c]	—
		Local effects on gastrointestinal tract	250 ppm, equal to 6.9 mg/kg bw per day	1250 ppm, equal to 35 mg/kg bw per day[c]
	12-month study of toxicity[d]	Systemic effects	8.4 mg/kg bw per day[c]	—
		Local effects on gastrointestinal tract	1.9 mg/kg bw per day	8.4 mg/kg bw per day[c]

[a] Diet
[b] Gavage
[c] Highest dose tested
[d] Capsules
[e] Two studies

Estimate of acceptable daily intake for humans

0–0.07 mg/kg bw

Estimate acute reference dose

0.3 mg/kg bw

Studies that would provide information useful for continued evaluation of the compound

Further observations in humans

Summary of critical end-points for propiconazole

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of oral absorption:	>80% in 48 h
Dermal absorption	About 30% in 10 h (rat)
Distribution:	Widely distributed; highest concentrations in the liver and kidney
Potential for accumulation:	Limited
Rate and extent of excretion:	>95% in the faeces and urine in 48 h; extensive enterohepatic recirculation (68% of administered dose in bile)
Metabolism in animals	Extensive; oxidation of propyl side-chain; hydroxylation of phenyl and triazole rings, plus conjugation; cleavage of dioxolane ring
Toxicologically significant compounds (animals, plants and the environment)	Propiconazole Triazolyl alanine and triazolyl acetic acid are produced in plants but not in animals
Acute toxicity	
Rat, LD_{50}, oral	1517 mg/kg bw
Rat, LD_{50}, dermal	>4000 mg/kg bw
Rat, LC_{50}, inhalation	>5 mg/l of air (4-h; nose only)
Rabbit, dermal irritation	Irritating
Rabbit, eye irritation	Not irritating
Skin sensitization	Sensitizing (Magnusson and Kligman study)
Short-term studies of toxicity	
Target/critical effect	Body weight, liver (mice, rats); erythrocytes (rat); stomach (dog)
Lowest relevant oral NOAEL	50 ppm, equal to 1.9 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	200 mg/kg bw per day (5 days/week)
Lowest relevant inhalation NOAEL	0.085 mg/l (6 h/day; 5 days/week)
Genotoxicity	Not genotoxic in vitro or in vivo
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Liver hypertrophy and tumours (mice) Liver, body weight, uterine lumen dilatation (rats)
Lowest relevant NOAEL	100 ppm, equal to 11 mg/kg bw per day (mice)
Carcinogenicity	Hepatocellular tumours in male mice (≥850 ppm, equal to 108 mg/kg bw per day). Phenobarbital-type mechanism. The NOAEL was 500 ppm (equal to 59 mg/kg bw per day). Unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Reproduction target/critical effect	Reduced pup weight at parentally toxic dose
Lowest relevant reproductive NOAEL	100 ppm, equivalent to 7 mg/kg bw per day (rat)
Developmental target/critical effect	Skeletal variations
Lowest relevant developmental NOAEL	30 mg/kg bw per day (rat)
Neurotoxicity/delayed neurotoxicity	No specific studies; no findings in other studies
Other toxicological studies	
Mechanism of induction of liver tumours	Phenobarbital-type mode of action indicated by cell proliferation, liver weight and microsomal enzyme induction patterns
Medical data	Local irritation associated with exposure to the formulated product

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.07 mg/kg bw	Rats, reproductive toxicity, pup and parental body weight	100
ARfD	0.3 mg/kg bw	Rats, developmental toxicity, embryo- or fetoxicity	100

References

Basler, W. & Gfeller, W. (1980) 28 Days cumulative toxicity study with CGA 64250 technical, on rats. Unpublished report, project No. 79/1659, dated 11 November 1980, from Ciba-Geigy Ltd, Experimental Toxicology Sisseln. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Bathe, R. (1978) Acute oral LD_{50} in the rat of technical CGA 64250. Unpublished report, project No. 78/5244 dated 7 December 1978, from Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Bathe, R. (1979a) Acute oral LD_{50} in the mouse of technical CGA 64250. Unpublished report, project No. 78/5243 dated 7 May 1979, from Ciba-Geigy Ltd, Experimental Toxicology, Sisseln. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Bathe, R. (1979b) Acute dermal LD_{50} in the rat of technical CGA 64250. Unpublished report, project No. 78/5245, dated January 22, from Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Beilstein, P. (1998) Propiconazole (CGA 64250) technical: effects on biochemical parameters in the liver following administration to male mice. Novartis Crop Protection Inc., Stein, Switzerland, Syngenta Unpublished report No. CB 97/22 (Syngenta No. CGA 64250/3359). Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Bissig, R. (1986) The metabolism of [U, 14C]phenyl CGA 64250 in mice after pre-treatment with unlabeled CGA 64250. Unpublished report No. 6/86 dated 20 May 1986 from Biochemistry Department R&D Plant Protection Agricultural Division, Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Bissig, R. (1992) Biliary excretion, absorption and distribution kinetics of [U-14C]phenyl CGA 64250 in the rat after oral administration. Ciba-Geigy Ltd, Basle, Switzerland, Unpublished report No. 6/86 dated 20 May 1986. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Borders, C.K., Salamon, C.M. & Mayhew, D.A. (1985) Two-generation reproduction study in albino rats. Unpublished report study No. 450,1202 dated 12 March, 1985 from Toxigenics, Inc. Decatur, Illinois. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Caresa, C. (1988) Dominant lethal study mouse (test for cytotoxic or mutagenic effects on male germinal cells). Addendum dated October 3, 1988 to Hool, G. & Müller, D. Ciba-Geigy. Unpublished report No. 790034 dated 31 October 1979. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Cresswell, D.G. & Hopkins, R. (1989) Absorption, distribution, metabolism and excretion of CGA 64250 in the rat. Unpublished report No. 380-105, dated 8 June 1989, from Hazelton Laboratories, UK. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Deparade, E. (1999) CGA 64250 technical, micronucleus test, mouse (OECD conform). Unpublished report No. CGA 64250/4268 from Novartis Crop Protection AG, Basel, Switzerland. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Deparade, E. & Arni, P. (1983) Salmonella/Mammalia microsome mutagenicity test. Induction of liver enzyme activity with Aroclor or with the test substance. Unpublished report No. 83/0121, dated 27 June 1983 from Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Doweyko, A.M. (1990a) Metabolism of [phenyl 14C]-propiconazole in chickens. Unpublished report No. F-00051, dated 14 June 1990, from Environmental Health Center, Farmington, USA for Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Doweyko, A.M. (1990b) Metabolism of phenyl ^{14}C-propiconazole in goats. Unpublished report No. F-00052, dated 31 July 1990 Environmental Health Center, Farmington, USA for Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Fisher, W.C. & Cassidy, J.E. (1980) Balance and metabolism of triazole, ^{14}C, CGA 64250 in a lactating goat. Unpublished report No. ABR, 80036 from Biochemistry Department Agricultural Division, Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Fritz, H. (1979) Report on CGA 64250 technical. Teratology study (seg. II) in rats. Unpublished report No. 790011 dated September 10, 1979 for Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Frölich, G., Bentley, P., Staubli, W. & Wachter, F. (1984) Promotion study with CGA 64250. Unpublished report, GU exploratory research project No. 83/4015, dated 18 December 1984, from Ciba-Geigy, Toxicology GU 2. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Fuchs, T. (1991) Epicutaneous test with propiconazole in 20 human volunteers. Unpublished report dated 1/8/1991 from Centre for Dermatology and Veneralogy, Hospital of the Georg-August University, Göttingen, Germany for Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Gerspach, R. (1997) Propiconazole (CGA 64250) Tech.: 18-month oncogenicity study in mice. Novartis Crop Protection AG, Stein, Switzerland. Unpublished report No. 943126 (Syngenta No. CGA64250/3142). Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Hambock, H. (1979) Distribution, degradation and excretion of CGA 64250 in the rat. Unpublished report No. 24/79 from Biochemistry Department R&D Plant Protection, Agricultural division, Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Hardisty, J. (1991) Long-term feeding study in mice with CGA 64250 (Propiconazole). Re-examination of the liver tumor response in male and female mice. Pathology report Unpublished HRC report No. CBG/196/81827 dated 6 May 1991 from Experimental Pathology Laboratories Inc., Research Triangle Park, NC, USA, Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Hartmann, H.R. & Gfeller, W. (1988) Acute aerosol inhalation toxicity in the rat. Unpublished report No. 871471 dated 14/1/1988 from Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Hool, G. & Müller, D. (1979) Dominant lethal study mouse (test for cytotoxic or mutagenic effects on male germinal cells). Unpublished report No. 790034 dated 31/10/1979 from Ciba-Geigy. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Hunter, B., Scholey, D. & Heywood, R. (1982a) CGA 64250 long-term feeding study in mice (final report). Unpublished report No. CBG/196/81827, dated 20 October 1982, from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Hunter, B., Scholey, D. & Heywood, R. (1982b) Potential tumorigenic and toxic effects in prolonged dietary administration to rats (final report). Unpublished report No. CBG 193/8284 (test No. 78/9023) from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Hunter, B., Slater, N., Gopinath, C. & Gibson, W. (1985) Potential tumorigenic and toxic effects in prolonged dietary administration to rats (addendum to final report); 25 June, 1985. Unpublished report No. CBG 193/821113/addendum from Huntingdon Research Centre, Huntingdon, England. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Jaquet, B. (1991) Industrial health record CGA 64250, Propiconazole. Unpublished report for Ciba-Geigy Ltd. from Medical surveillance, Monthey, Switzerland. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Johnson, W.D., Thompson, S.W. & Becci, P.J. (1985) One year subchronic oral toxicity study in beagle dogs with CGA 64250 technical (final report). Unpublished report No. 7737, dated 28 May 1985, from Food & Drug Research Laboratories Inc. Waverly, NY, USA. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Khera, K.S. (1985) Maternal toxicity: A possible etiological factor in embryo-fetal deaths and fetal malformations of rodent – rabbit species. *Toxicology*, **31**, 129–153.

Madrid, V. & Cassidy, J.E. (1981) Characterization of metabolites in urine, milk and liver of a goat treated with triazole, 14C-CGA, 64250 Unpublished report No. ABR, 81007, dated 27 March 1981, from Biochemistry Department, Agricultural Division, Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Mallows, S., Levy, E., Giknis, M.L.A. & Yau, E.T. (1987) A modified teratology (segment II) study in albino rats with CGA 64250. Unpublished report No. 96189, dated 6 February 1987, from Ciba-Geigy Pharmaceutical Division, NJ, USA. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Marcsisin, J.F., Wimbet, K.V., Giknis, M.L.A., Arthur, A.T. & Yau, E.T. (1987) A teratology (segment II) study in rats. Unpublished report No. 86004, dated 28 January 1987, from Ciba-Geigy Pharmaceutical Division, NJ, USA. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Mücke, W. (1979) Characterization of urinary and faecal metabolites of rats after oral application of CGA 64250. Unpublished report No. 356/79 from Biochemistry Department, R&D Plant Protection, Agricultural Division, Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Mücke, W. (1983) The metabolism of CGA 64250 in the rat. Unpublished report No. 245/83, dated 1 September 1983, from Biochemistry Department, R&D Plant Protection, Agricultural Division, Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Murphy, T., Brown, K., Doornheim, D. & Marco, G. (1986) Dermal absorption of ^{14}C-propiconazole in rats after a ten hour exposure period. Unpublished report No. ABR-86053, dated 8/4/1986, from Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Potrepka, R.F. & Turnier, J.C. (199la) Subchronic dietary toxicity study with CGA, 64250 in mice. Unpublished laboratory report No. F,00098, dated 30 April 1991, from Ciba-Geigy Corporation, Farmington, CT, USA. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Potrepka, R.F. & Turnier, J.C. (1991b) 13-week dietary toxicity study with CGA 64250 in male mice. Unpublished laboratory report No. F,00197, dated 30 April 1991, from Ciba-Geigy Corporation, Farmington, CT, USA. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Puri, E. & Muller, D. (1982) Autoradiographic DNA repair test on rat hepatocytes CGA 64250. Unpublished report No. 81/1514, dated 12 August 1982, from Ciba-Geigy. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Raab, D.M., Youreneff, M.A., Giknis, M.L.A., Arthur, A.T. & Yau, E.T. (1986) A teratology study (segment II) in New Zealand White rabbits. Unpublished report No. MIN 852172, dated 1/8/1986, from CGS – Ciba-Geigy Corporation, Summit NJ, USA. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Sachsse, K., Suter, P., Luetkemeier, H., Zak, F. & Hess, R. (1979a) Three months toxicity study on rats of CGA 64250 technical. Unpublished report No. 79/0014, dated 30 August 1979, from Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Sachsse, K., Ullman, L., Luetkemeier, H., Zak, F. & Hess, R. (1979b) 21-Day percutaneous toxicity study in rabbits with technical CGA 64250. Unpublished report No. 79/0007 from Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Sachsse, K., Ullman, L., Luetkemeier, H., Zak, F. & Hess, R. (1980a) 90-Days aerosol inhalation study on rats with technical CGA 64250. Unpublished report No. 79/0006, dated 10 September 1980, from Ciba-Geigy Ltd, Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Sachsse, K., Bathe, R., Luetkemeier, H., Zak, F. & Hess, R. (1980b) 3-Month toxicity study with CGA 64250 on dogs. Unpublished report No. 78/5751, dated 9 August 1980, from Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Schaetti, P. (1999) 18 Month reference study in CD-1 mice (control diet): reference control data. Sub-report in pathology concerning neoplasia. Unpublished report No. 960024 from Novartis Crop Protection AG, Stein, Switzerland. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Seim, V.W. & Brown, G. (1984) Biological report for the metabolism of phenyl and triazole, 14C, CGA, 64250 in laying hens. Unpublished report No. BIOL,83011, dated 6/1/1984, from Agricultural division, Ciba-Geigy Corporation, Vero Beach, Florida. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Simoneaux, B. (1983) Dermal absorption of triazole, 14C, CGA, 64250 by rats. Unpublished report No. ABR,82068 from Biochemistry Department Agricultural Division, Ciba-Geigy Corporation, Greensboro, N.C. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Sommer, E. (1999) Skin sensitisation in the guinea pig (maximisation test); CGA 64250. Unpublished report No. 993101, dated 7 September 1999, for Novartis Crop Protection AG. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Strasser, F. & Arni, P. (1984) Chromosome studies on human lymphocytes in vitro. Unpublished report No. 840025, dated 10/5/1984, for Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Strasser, F. & Arni, P. (1987) Micronucleus test (Chinese hamster). Unpublished report No. 860359, dated 14/12/1987, for Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Strasser, F.F. & Muller, D. (1982a) L5178Y/TK+/, mouse lymphoma mutagenicity test. CGA 64250. Unpublished report No. 81/1516, dated 10 August 1982, from Ciba-Geigy, Protection of Health and Environment. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Strasser, F.F. & Muller, D. (1982b) BALB/3T3 cell transformation assay CGA 64250. Unpublished report No. 79/0806, dated 10 August 1982, from Ciba-Geigy. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Szolics, I.M. & Simoneaux, B.J. (1985) Distribution, excretion and partitioning characteristics of phenyl and triazole labeled propiconazole in chickens. Unpublished report No. ABR-85043, dated 25/6/1985, from Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Ullmann, L. (1978a) Skin irritation in the rabbit after single application of technical CGA 64250. Unpublished report No. 78/5249, dated 26 October 1978, from Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Ullmann, L. (1978b) Eye irritation in the rabbit after single application of technical CGA 64250. Unpublished report No. 78/5248, dated 26 October 1978, from Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Ullmann, L. (1979a) Acute dermal LD_{50} in the rabbit of technical CGA 64250. Unpublished report No. 79/0375, dated 2 July 1979, from Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd. Basle, Switzerland.

Ullmann, L. (1979b) Skin sensitizing (contact allergenic) effect in guinea pigs of technical CGA 64250. Unpublished report No. 78/5250, dated 8 February 1979, from Ciba-Geigy Ltd. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Waechter, F., Bentley, P. & Staubli, W. (1984) The effect of propiconazole on drug metabolizing enzymes in the livers of male rats and mice. Unpublished report dated July 1984. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Weber, E. (1997) Propiconazole (CGA 64250/3139): assessment of hepatic cell proliferation in the course of an 18-month oncogenicity study in male mice. Unpublished report No. CB 96/42 (CGA 64250/3139) from Novartis Crop Protection AG. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

Weber, E. (1999) Propiconazole (CGA 64250/4200): assessment of hepatic cell proliferation in male mice. Unpublished report No. CB 97/23 (CGA 64250/4200) from Novartis Crop Protection AG. Submitted to WHO by Syngenta Ltd, Basle, Switzerland.

TRIADIMENOL AND TRIADIMEFON

First draft prepared by
Jürg Zarn,[1] Les Davies[2] and Alan Boobis[3]
[1] Food Toxicology Section, Swiss Federal Office of Public Health, Zurich, Switzerland;
[2] Science Strategy and Policy, Office of Chemical Safety, Australian Government Department of Health and Ageing, Woden, ACT, Australia; and
[3] Experimental Medicine & Toxicology, Division of Medicine, Faculty of Medicine, Imperial College London, London, England

Explanation

The toxicity of triadimenol ((1RS,2RS;1RS,2SR)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-ol), a triazole fungicide, was evaluated by the 1989 JMPR, when an acceptable daily intake (ADI) of 0–0.05 mg/kg bw was established based on a no-observed-adverse-effect level (NOAEL) of 5 mg/kg bw per day in a two-generation study in rats. As currently manufactured, triadimenol is an 80 : 20 mixture of the diastereoisomers A (1RS,2SR) and B (1RS,2RS). Older studies of toxicity in the database were performed with 60 : 40 mixtures.

Triadimefon is closely chemically related to triadimenol, with which it shares some similar metabolic pathways in animals. The toxicity of triadimefon ((RS)-1-(4-chlorophenoxy)-3,3-dimethyl-1-(1*H*-1,2,4-triazol-1-yl)butan-2-one) was evaluated by the JMPR in 1981, 1983 and 1985. An ADI of 0–0.03 mg/kg bw was established based on a NOAEL of 50 ppm, equivalent to 2.5 mg/kg bw per day, in a 2-year dietary study in rats.

Although triadimenol and triadimefon are independent active ingredients, on the basis of their close chemical and toxicological relationship they were re-evaluated together by the present Meeting within the periodic review programme of the Codex Committee on Pesticide Residues. Triadimenol and triadimefon act as systemic fungicides by blocking fungal ergosterol biosynthesis. The mechanism of action of these fungicides is inhibition of demethylation.

TRIADIMENOL

Evaluation for acceptable daily intake: triadimenol

1. Biochemical aspects

1.1 Absorption, distribution and excretion

The metabolism and the excretion of [phenyl-UL-^{14}C]triadimenol in Sprague-Dawley rats was investigated in a pre-good laboratory practice (GLP) study and no statement of quality assurance (QA) was provided. To study the pattern of excretion, two male and two female rats were given either isomer A or isomer B (specific activity, 19.8 mCi [732.6 MBq]/mmol) as a single dose at 4 mg/kg bw by gavage. For isomer A, urine and faeces were collected at 4, 8, 12 and 24 h and then daily until termination on day 6. For isomer B, collection times of 4 h and 8 h were replaced by a time-point at 6 h. Samples of expired air, blood and organs were collected for analysis of radiolabel. For tissue and metabolite analyses, 10 animals of each sex were given [^{14}C]phenyl-labelled triadimenol A (9.9 mCi [366.3 MBq]/mmol) at a dose of 25 mg/kg bw by gavage. At 1, 2, 4, 8 and 24 h after administration, two animals per sex were killed and samples of blood and organs were collected for radiolabel analyses.

No radioactivity was detected in the expired air. In the faeces, males excreted 55% of the administered dose of isomer A and 78% of isomer B, while females excreted 37% and 44%, respectively. In the urine, males excreted 31% and 14%, and females excreted 52% and 47% of the administered dose of isomer A and B, respectively. Thus, males in particular eliminated a significantly greater proportion of isomer B than isomer A in the faeces. Maximal residue concentrations in organs and blood were <0.01 ppm, with the exception of the liver, which contained residue at 0.01–0.06 ppm. Tissue concentrations peaked at 1–4 h, with highest concentrations in fat, skin, liver and kidney. The estimated average elimination half-life for the radiolabel was 24 h for both isomers in both sexes.

The pattern of metabolites was not affected by the sex of the animals. Major metabolic reactions were hydroxylation of one of the *t*-butyl methyl groups of triadimenol with subsequent oxidation to the carboxylic acid. Additionally, limited oxidation of triadimenol to triadimefon was observed. Conjugate formation was of minor significance. Particularly in males, unchanged isomer B was excreted to a greater extent than was unchanged isomer A (Puhl & Hurley, 1978).

In a whole-body autoradiography study, male Wistar rats were given [phenyl-UL-^{14}C]triadimenol (specific activity, 29.2 mCi/mmol i.e. 3.66 MBq/mg]; A : B = 80 : 20) as a single dose at 3 mg/kg bw by gavage. Expired air, urine and faeces were collected individually and single animals were killed at intervals of between 1 h and 168 h after dosing. This study complied with the Organisation for Economic Co-operation and Development (OECD) requirements for GLP. Triadimenol was absorbed rapidly and peak conecntrations were reached at 1 h in most organs. In the eyes and the urinary bladder, peak concentrations were reached at 4 h and 8 h, respectively. Within 24 h after dosing, 79–90% of the radioactivity was excreted, and excretion was virtually complete by 96 h. Only 0.01% of the administered dose was expired. Radiolabel was found (in decreasing order of activity) in perirenal and brown fat, urinary bladder, liver, adrenals, lachrymal glands, kidney and nasal mucosa. These activities corresponded to concentrations of between 2.4 and 0.4 µg/g. At 120 h after administration, elimination from most organs resulted in concentrations of triadimenol that were below the limit of quantification or detection (Justus, 2002a). In a study of biokinetics and metabolism, groups of male and female Wistar rats were given [phenyl-UL-^{14}C]triadimenol (specific activity, 29.2 mCi/mmol i.e. 3.66 MBq/mg; A : B = 80 : 20) as a single oral dose at 1 mg/kg bw. Additional groups dosed in this way included a group of male rats that had been pretreated with unlabelled triadimenol for 14 days before dosing, and a group of bile-duct cannulated male rats. A group of male rats was given a single dose of [phenyl-UL-^{14}C]triadimenol at 100 mg/kg bw. Another group of male rats received single low doses of [phenyl-UL-^{14}C]triadimenol as individual isomers A and B at 1 mg/kg bw. All test groups consisted of four animals (except the group of bile-duct cannulated rats, which comprised six animals) and the duration of investigation was 120 h. In these studies, radioactivity was measured in urine, faeces, plasma, organs and the whole body after termination. This study complied with OECD requirements for GLP. Triadimenol was rapidly absorbed, with peak plasma concentrations occurring at 1.5 h. The elimination half-lives of the radiolabel were in the range of 6 h to 15 h, with the longest half-lives for males at the high dose and females at the low dose. In animals at the low dose, males and females excreted 14–21% and 48% of the dose via urine, respectively. The rest of the administered dose was excreted in the faeces, with very low levels remaining in the whole body (0.02–0.06% of the administered dose). In bile-duct cannulated males, 6% of the radiolabel was recovered in the urine and 93% in the bile, indicating enterohepatic recycling. Pretreatment of the animals with unlabelled triadimenol for 14 days did not affect the results (Justus, 2002b).

In a study of dermal absorption, groups of 24 Charles River Crl:CD rats were given ^{14}C ring-labelled triadimenol (15.78 mCi [583.9 MBq]/mmol per l) at a dose of 0.01, 0.1, 1.0 or 10 mg (equivalent to 0.04–40 mg/kg bw) applied to an area of shaved skin of 15 cm^2. The test material was covered by a gauze patch after application. Four animals per group were bled and then terminated at 0.5, 1, 2, 4, 8 and 24 h after dosing. Urine and faeces were collected from each animal. Radioactivity was also determined in whole carcass, skin wash and excised skin. Dermal absorption was rapid. It was estimated that 50% of the test material administered was absorbed, and that absorption was somewhat slower at higher concentrations (with half-lives for elimination of radiolabel ranging from 27 h at the lowest dose to 86 h at the highest dose), suggesting that transport was saturated (Leeling et al., 1988, quoted from JMPR 1989).

1.2 Metabolism

Triadimenol was extensively metabolized (see Figure 1), mainly by oxidation of one of the *t*-butyl methyl groups to the hydroxyl or carboxy group. Non-conjugated metabolites predominated in the urine and the faeces, while bile metabolites were extensively glucuronidated. The most abundant metabolite in the urine and faeces was carboxytriadimenol (KWG 1640 [M02], 45–64% of the administered dose), followed by hydroxy triadimenol (KWG 1342 [M10], 18–33% of the administered dose). Levels of each of the other metabolites were <8% of the administered dose. The most abundant metabolite in the bile was hydroxytriadimenol glucuronide (M23, 52% of the administered dose), followed by triadimenol glucuronide (M24, 21% of the administered dose) and carboxytriadimenol glucuronide (M30, 17% of the administered dose), respectively. Levels of each of the other metabolites were <3% of the administered dose. Cleavage of the triazole or 4-chlorophenyl moiety was found only to a minor extent, producing triadimenol-ketocarboxyglucuronide (M34) and a conjugate of 4-chlorophenol (M07) (maximum, 3% and 0.5% of the administered dose, respectively). Female rats excreted more carboxytriadimenol in the urine than did males, but less in the faeces (Justus, 2002b).

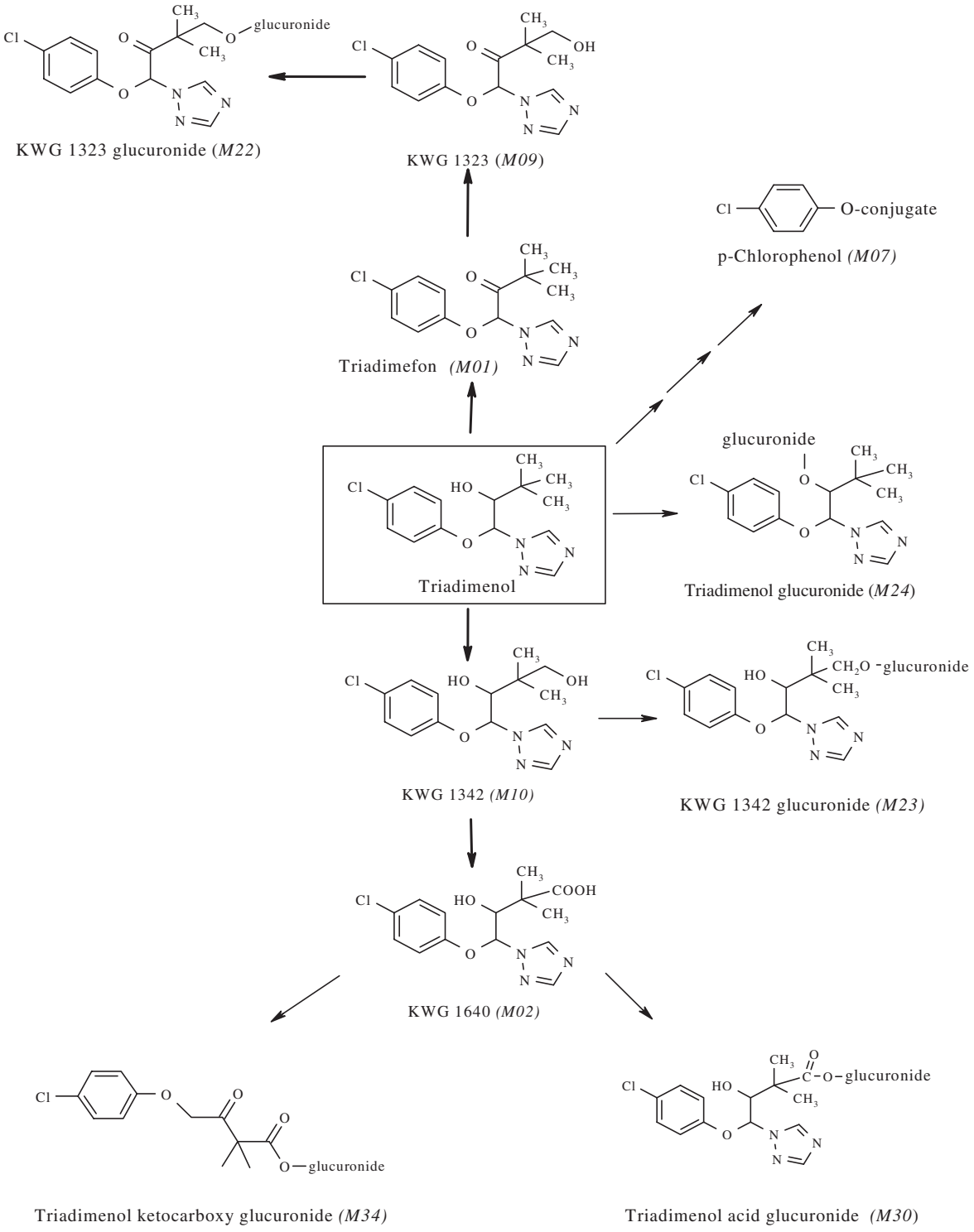
1.3 Effects on enzymes and other biochemical parameters

In a comparative assay for enzyme inhibition in vitro, the effects of triadimenol and triadimefon and other pesticides on aromatase activity were studied. Aromatase converts androgens to estrogens and is therefore important for the balance of sex steroids. Half maximal inhibition of human placental microsomal aromatase was observed for triadimenol and triadimefon at 21 µmol/l and 32 µmol/l, respectively. This was judged to be weak inhibition when compared with that of other azole compounds such as prochloraz (Vinggaard et al., 2000).

Both compounds were found to be weak agonists of the estrogen receptor in MCF7 breast cancer cells (triadimefon and triadimenol at 10 µmol/l induced a 2.4 and 1.9-fold increase in cell proliferation, respectively) but not in estrogen receptor α-transfected yeast cells. Other azole compounds, such as prochloraz and imazalil, were either negative in these assays or had very low activity (Vinggaard et al., 1999).

In a review of azole compounds including triadimenol and triadimefon, a possible relationship between inhibition of mammalian lanosterol demethylase and aromatase and developmental and reproductive effects in laboratory animals was considered (Zarn et al., 2003).

Figure 1. Proposed metabolism of triadimenol in rats



From Bayer CropScience AG (2003); the scheme is reproduced from the manufacturer's evaluation.

2. Toxicological studies

2.1 Acute toxicity

The acute toxicity of triademenol is summarized in Table 1.

(a) General toxicity

In the studies of acute toxicity, signs of intoxication in rats included impairment of the general health condition, piloerection, drowsiness, laboured breathing, cramps, decreased and increased mobility, aggressiveness, self-mutilation and lying on their sides (Thyssen & Kimmerle, 1976b; Mihail & Thyssen, 1980). With the exception of subcutaneous application in mice, there was very little difference in acute toxicity between the sexes; the reason for this is not known. In a study of acute oral toxicity in rats, the median lethal dose (LD_{50}) of isomer A was an order of magnitude lower than that of isomer B. Since the vehicle was the same in both studies (Cremophor EL), stereochemical differences in kinetics and metabolism might explain this finding. A study with the isomer composition A:B = 80:20 suggests an influence of feeding status on the acute oral toxicity of triadimenol.

(b) Dermal and ocular irritation

In New Zealand White rabbits and in a unspecified rabbit strain, incidental and not treatment-related very slight dermal and ocular irritation reactions were observed in a few

Table 1. Acute toxicity of triadimenol

Isomer ratio	Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Purity (%)	Reference
A:B = 60:40	Mouse	NMRI	Male	Oral	1300		NS	Thyssen & Kimmerle (1976b)
	Mouse	NMRI	Female	Oral	1267			
	Mouse	NMRI	Male	Subcutaneous	1580			
	Mouse	NMRI	Female	Subcutaneous	2441			
	Rat	Wistar	Male	Oral	1161			
	Rat	Wistar	Female	Oral	1105			
	Rat	Wistar	Male	Intraperitoneal	367			
	Rat	Wistar	Female	Intraperitoneal	352			
	Rat	Wistar	Male	Dermal	>5000			
	Rat	Wistar	Female	Dermal	>5000			
	Rat	Wistar	Males and females	Inhalation		>0.315		
Isomer A	Rat	NS	Male	Oral	579		99.9	Flucke (1979a)
Isomer B	Rat	NS	Male	Oral	5000		99.0	Flucke (1979b)
A:B = 60:40	Rat	NS	Male	Oral (not stated whether fed or fasted)	819–895		NS	Flucke (1979c)
A:B = 80:20	Rat	Wistar	Male	Oral (fasted)	689		92.4	Mihail & Thyssen (1980)
			Female	Oral (fasted)	752			
	Rat	Wistar	Male	Oral (fed)	1098			
			Female	Oral (fed)	1037			
	Rat	Wistar	Male	Intraperitoneal (fed)	371			
			Female	Intraperitoneal (fed)	286			
	Rat	Wistar	Males and females	Dermal	>5000			
	Rat	Wistar	Males and females	Inhalation (4 h)		>0.954		

NS, not stated

cases. In humans, there was no evidence of primary skin irritation (Thyssen & Kimmerle, 1976b; Mihail & Thyssen, 1980; Nagashima, 1982a; Nagashima, 1982b; Kroetlinger, 1993).

(c) Dermal sensitization

In a Magnusson-Kligman maximization test, 10 male and 10 female guinea-pigs were treated intradermally with 0.1 ml of a 2.5% formulation of triadimenol and, additionally, 1 week later by topical application of a 25% formulation of triadimenol. The challenge with a 25% formulation was performed 2 weeks after the topical application. Triadimenol was not a skin sensitizer (Flucke, 1981).

2.2 Short-term studies of toxicity

Mice

Groups of 10 male and 10 female Crl:CD-1(ICR)BR mice were fed diets containing triadimenol (purity, 97.4%; A:B = 80:20) at a concentration of 0, 160, 500, 1500 or 4500 ppm for 13 weeks. The average daily intakes of triadimenol were 0, 24.9, 76.8, 235 and 872 mg/kg bw per day for males and 0, 31.4, 94.1, 297 and 797 mg/kg bw per day for females. This study complied with OECD requirements for GLP. A slightly increased rate of mortality was observed in males at 4500 ppm. In this group, the males also showed higher intake of food, piloerection and squatting position. Mean body weights were reduced at 1500 ppm in males and at 4500 ppm in females. Erythrocyte volume fraction was decreased and mean corpuscular haemoglobin concentration was increased in females at 4500 ppm. Lower leukocyte counts were found in males receiving triademenol at concentrations of ≥1500 ppm. Hypertrophy of liver cells was found in males given triademenol at ≥500 ppm and in females at ≥1500 ppm, with increased liver weights, cytoplasmic vacuolation and single cell necrosis at ≥1500 ppm in both sexes. These liver effects were accompanied by several changes in clinical chemistry. In animals of both sexes, increased activity of aspartate and alanine aminotransferases (AST and ALT) and glutamate dehydrogenase at ≥500 ppm was observed (Table 2). At ≥1500 ppm, decreased total protein concentrations, increased triglyceride concentrations and lipid storage were found in both sexes. In males at ≥1500 ppm and in females at 4500 ppm, decreased albumin and cholesterol concentrations were observed. Bilirubin concentrations were decreased at dietary concentrations of of ≥500 ppm in males and ≥1500 ppm in females. In both sexes, hepatic aminopyrine-*N*-demethylase activity was

Table 2. Enzyme activities in mice fed with diets containing triademenol for 13 weeks

Dietary concentration (ppm)	AST (U/l)	ALT (U/l)	Glutamate dehydrogenase (U/l)
Males			
0	26.0	29.7	5.8
160	31.1	31.7	13.6*
500	39.2**	47.3*	46.0*
1500	56.3**	75.9**	78.1**
4500	77.7**	158.1**	84.3**
Females			
0	30.2	27.9	9.1
160	34.1	30.5	9.6
500	41.3*	38.4*	26.1*
1500	88.4*	85.8**	81.1**
4500	133.1*	218.6**	110.4**

From Schladt & Sander (1998)
ALT, alanine aminotransferase; AST, aspartate aminotransferase
* $p = 0.05$
** $p = 0.01$

increased at ≥160 ppm. In animals of both sexes at 4500 ppm, adrenal weights were increased, and in females the adrenal cortical X-zone was lacking vacuoles; in mice, the X-zone of the adrenals usually shows vacuoles with a strain dependent incidence—the significance of this finding is not clear, neither is the role of the X-zone in general). The significance of this finding was unresolved (the study author concluded that this was not an unusual finding, being seen after dosing with several compounds from different chemical classes). The NOAEL was 500 ppm, equal to 76.8 mg/kg bw per day, on the basis of microscopic changes in the liver and clinical chemistry findings at 1500 ppm (Schladt & Sander, 1998).

Rats

Groups of 20 male and 20 female Wistar rats were given technical-grade triadimenol (purity, 98.5%; A : B = 60 : 40) at a dose of 0, 1.5, 5, 15 and 45 mg/kg bw per day by gavage for 28 days. One-half of all the animals in each group was then terminated and the remaining animals were kept for another 28 days as a recovery group. This is a pre-GLP study and no statement on QA was provided. In females, the only noteworthy findings were increased absolute (50 mg in the control group, 57, 57 and 60 mg at the lowest, intermediate and highest dietary concentrations) and relative (28 mg in the control group, 33, 32 and 33 mg at the lowest, intermediate and highest dietary concentrations) ovary weight in all treated groups at the end of the dosing period, but not at the end of the recovery period. These effects on ovary weight were without an apparent dose–response relationship and were judged to be incidental. In males at the highest dose only, there was minimally but significantly increased absolute and relative weights of the thyroid. No other treatment-related changes were observed regarding behaviour, appearance, weight gain, blood chemistry, liver and kidney functions, organ weights, gross pathology and histopathology. The NOAEL was 45 mg/kg bw per day, the highest dose tested (Thyssen & Kaliner, 1976).

To study the toxicological effects of the two different isomer compositions, groups of 20 male and 20 female Wistar rats were given either technical-grade triadimenol (A : B = 80 : 20; purity, 98.3%) at 15, 45 or 100 mg/kg bw per day, or technical-grade triadimenol (A : B = 60 : 40; purity, 84.7%) at 45 or 100 mg/kg bw per day. Half of the animals of each group were terminated and the remaining animals were kept for another 28 days as a recovery group. This is a pre-GLP study and no statement on QA was provided. No treatment-related changes were observed regarding appearance, weight gain, blood chemistry, liver and kidney functions, gross pathology or histopathology. The only effects observed with both isomer compositions were slightly and transiently increased liver weights in females at the highest dose. Additionally, at doses of ≥45 mg/kg bw per day with both batches very minor induction of hepatic *N*- and *O*-demethylases and cytochrome P450 was found in both sexes. However, these changes lacked a clear dose–response relationship. At doses of ≥45 mg/kg bw per day, a slightly increased motor activity was observed with both isomer mixtures in both sexes, persisting for the first 2 h after dose application. On the basis of the slight behavioural changes observed at 45 mg/kg bw per day with both mixtures, the NOAEL for the triadimenol isomer composition A : B = 80 : 20 was 15 mg/kg bw per day and for A : B = 60 : 40 it was <45 mg/kg bw per day (Mihail & Vogel, 1981).

Groups of 15 male and 15 female Wistar rats were fed diets containing technical-grade triadimenol (purity, 98%; A : B = 60 : 40) at a concentration of 150, 600 or 2400 ppm for 3 months. The control group consisted of 30 animals of both sexes. Average daily compound intakes were 12.2, 49.2 and 203 mg/kg bw per day for males and 17.1, 71.3 and 287 mg/kg bw per day for females. This is a pre-GLP study and no statement of QA was

provided. Behaviour, appearance and survival were not affected in any group. At the highest dose, body-weight gain was slightly but significantly reduced in both sexes, while liver weights in both sexes and kidney and ovary weights were significantly increased in females. However, histopathology on all organs did not indicate any damage. At the highest dose, minor changes in blood parameters consisted of reduced erythrocyte volume fraction, and relative eosinophil counts in females and reduced mean corpuscular volume and mean corpuscular haemoglobin in males. The NOAEL was 600 ppm, equal to 49.2 mg/kg bw per day, on the basis of minor changes in body weight and organ weights and effects on blood parameters at 2400 ppm (Loeser & Kaliner, 1977).

Groups of 20 male and 20 female Sprague-Dawley Crj:CD rats were fed diets containing technical-grade triadimenol (purity, 94%; A:B = 80:20) ata a concentration of 0, 120, 600 or 3000 ppm for 3 months. The average daily intakes of triadimenol were 0, 8.0, 39.6 and 209 mg/kg bw per day for males and 0, 9.4, 46.4 and 221 mg/kg bw per day for females. There was no report on compliance of this study with any GLP standards and no statement of QA was provided. At the highest dietary concentration, piloerection and depilation were observed in animals of both sexes in the first month of exposure. Body-weight gain was reduced in both sexes, concomitant with initial (males) and continuing (females) reduced food intake and initially reduced food efficiency. At dietary concentrations of ≥600 ppm, absolute and relative liver weights were increased and the livers were enlarged in both sexes. Additionally, the livers of females showed a pronounced lobular structure and in both sexes at the highest dietary concentration, histopathology on the liver revealed fatty changes and eosinophilic degeneration of hepatocytes. At the highest dietary concentration, haemoglobin and erythrocyte volume fraction were reduced in both sexes. Clinical chemistry showed reduced triglyceride and free fatty acid concentrations in both sexes at the highest dietary concentration and females had increased total cholesterol, phospholipid and total protein concentrations and a decreased albumin/globulin (A/G) ratio and decreased albumin concentrations.

The NOAEL was 600 ppm in the diet, equal to 39.6 mg/kg bw per day, on the basis of effects on the liver observed at 3000 ppm (Nishimura, 1983).

Groups of 10 male and 10 female Wistar rats were exposed to technical-grade triadimenol (purity not stated) at a concentration averaging 0.030, 0.068 or 0.229 mg/l as a liquid aerosol in ethanol/polyethylene glycol solvent by inhalation for 6 h per day for 3 weeks. Measurements were gravimetric and concentrations reported are actual rather than nominal. Animals in the control group were exposed only to the ethanol/polyethylene glycol solvent. This is a pre-GLP study and no statement on QA was provided.

No treatment-related findings were recorded in behaviour, appearance, body and organ weights, clinical chemistry, haematology or histopathology.

The NOAEC was 0.229 mg/l, the highest concentration tested (Kimmerle, 1976).

Rabbits

Groups of six male and six female New Zealand White rabbits were given triadimenol (purity, 98%; A:B = 80:20) at a dose of 0, 50 or 250 mg/kg bw per day applied dermally on either intact or abraded skin for 6 h per day, 5 days per week, for 3 weeks. There was no report on compliance with any GLP standards and no statement of QA was provided.

No treatment-related findings related to behaviour, appearance, body and organ weights, clinical chemistry, haematology or histopathology were recorded.

The NOAEL was 250 mg/kg bw per day, the highest dose tested (Heimann & Schilde, 1984).

Dogs

Groups of four male and four female beagle dogs were fed diets containing technical-grade triadimenol (purity, 98.5%; A : B = 60 : 40) at a concentration of 0, 150, 600 or 2400 ppm for 13 weeks. The average daily intakes of triadimenol were 0, 4.5, 17.8 and 71 mg/kg bw per day (calculated from the daily intakes per animal of 0, 44.5, 178.8 and 709.5 mg and a default body weight of 10 kg. This is a pre-GLP study and no statement of QA was provided.

The body-weight gain of animals at the highest dose was slightly reduced (statistically not significant) and the relative weights of their liver and kidneys (males only) were increased. Alkaline phosphatase (ALP) activity was elevated in all treated animals without any dose–response relationship. At the highest dose, cytochrome P450 levels and aminopyrine *N*-demethylating activity were increased. At 6 weeks, but not at the end of the study, animals at ≥600 ppm showed increased serum glutamate transaminase activity and cholesterol concentrations. At the end of the study, elevated concentrations of cholesterol were statistically significant only at the highest dose.

The NOAEL was 600 ppm, equivalent to 17.8 mg/kg bw per day, on the basis of effects on organ weightss and changes in clinical chemistry parameters at 2400 ppm (Hoffmann & Kaliner, 1977).

Groups of six male and six female beagle dogs were fed diets containing technical-grade triadimenol (purity, 98%; A : B = 80 : 20) at a concentration of 0, 10, 30 or 100 ppm for 6 months. The average daily intakes of triadimenol were 0, 0.4, 1.2 and 4 mg/kg bw per day (calculated from the daily intakes per animal of 0, 4.03, 12.26 and 40.2 mg and a default body weight of 10 kg). There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

There were no treatment-related findings related to appearance, behaviour, ophthalmology, clinical chemistry, haematology or gross pathology. No microscopic histopathology was performed on the organs.

The NOAEL was 100 ppm, the highest concentration tested, equivalent to 4.0 mg/kg bw per day (Hoffmann, 1984).

Groups of four male and four female beagle dogs were fed diets containing technical-grade triadimenol (purity, 94.9%; A : B = 60 : 40) 0, 150, 600 and 2400 ppm for 2 years. The average daily intakes of triadimenol were 0, 5.6, 21.1 and 85.9 mg/kg bw per day (calculated from the daily intakes per animal of 0, 55.7, 211.3 and 859.0 mg and a default body weight of 10 kg). There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

The control group showed an unusually large body-weight increase. Therefore, the body-weight gains in all groups deviated considerably from the control group in a non-dose-

related manner. The study author concluded that there were no treatment-related body weight effects. At the highest dose, cytochrome P450 levels and aminopyrine *N*-demethylating activity were increased. The NOAEL was 600 ppm, equivalent to 21.1 mg/kg bw per day, on the basis of clinical chemistry changes at 2400 ppm (Hoffmann & Vogel, 1984).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 50 male and 50 female CF_1/W 74 mice were fed diets containing triadimenol (purity, 95%; A : B = 60 : 40) at a concentration of 0, 125, 500 or 2000 ppm for 2 years. The average daily intakes of triademenol were 0, 30, 140 and 620 mg/kg bw per day for males and 0, 50, 200 and 810 mg/kg bw per day for females. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

Males and females at ≥500 ppm showed reduced body-weight gain, which was statistically significant at the end of the study only for males at the highest dose. Food consumption was not affected. At the highest dose, the absolute and relative weights of the liver of males and females were increased, as were the weights of testes in males. The weights of ovaries were not reported. The only findings in haematology were Jolly bodies in all females of all dosed groups and in one male in each of the groups at 125 and 500 ppm and in nine males at 2000 ppm. This observation could not be explained and was judged by the study author to be of no biological significance. At 24 months, ALT and AST activities were increased in each sex at ≥500 ppm, as was ALP at 2000 ppm (Table 3). After 12 months at the highest dose, cholesterol concentrations were decreased in both sexes. At the end of the study, this effect was only significant for males at the highest dose. At 2000 ppm, the livers of both sexes showed more hyperplastic nodules and females in the groups receiving the intermediate and highest doses had increased incidences of adenomas (intermediate dose: 4 (8%); and highest dose: 6 (12.2%); versus 0 in the control group) (Table 3). In studies of historical controls, liver adenomas were found in 3.9% (mean value from 14 studies) of females, with a range of 0–12%. It is therefore questionable whether triademenol has carcinogenic potential. In two males at the highest dose, a carcinosarcoma in the urinary bladder was identified. Additionally, a slightly increased incidence of cystic alterations was found in the thyroid (more pronounced in males).

Table 3. Liver enzyme activities and incidence of liver adenomas in mice fed diets containing triademenol for 2 years

Dietary concentration (ppm)	AST (U/l)	ALT (U/l)	Adenoma
Males			
0	37.5	53.7	6
125	54.0	71.4	4
500	58.2	88.0*	5
2000	97.8**	159.4**	8
Females			
0	40.0	30.7	0
125	48.6	42.8	0
500	53.6*	48.0*	4
2000	185.7**	283.5**	6

From Bomhard & Loeser (1982)
ALT, alanine aminotransferase AST, aspartate aminotransferase
*$p = 0.05$
**$p = 0.01$

The NOAEL was 500 ppm, equal to 140 mg/kg bw per day, on the basis of increased incidence of adenoma at 2000 ppm (Bomhard & Loeser, 1982).

Groups of 50 male and 50 female Crl:CD-1(ICR)BR mice were fed diets containing triadimenol (purity, 96.8%) at a concentration of 0, 80, 400 or 2000 ppm for 80 weeks. The average daily intakes of triadimenol were 0, 11.3, 60.2 and 340 mg/kg bw per day for males and 0, 17.2, 91.3 and 472 mg/kg bw per day for females. This study complied with OECD requirements for GLP.

Males and females at 2000 ppm had reduced body-weight gain (16% in males, 12% in females) and males of this group had slightly increased feed intake. At the highest dose, absolute and relative liver weights of males and females and absolute and relative brain weights of males were increased. Statistical significant increases in hepatocellular hypertrophy and single cell necrosis were found in males at ≥400 ppm and hepatocellular hypertrophy was found in females at 2000 ppm (Table 4). Males and females at 2000 ppm showed more yellow-brown pigmentation in the liver, while females of this group also had fatty changes and intracytoplasmic hepatocellular vacuolation and males had increased inflammatory infiltration, respectively. At doses of ≥80 ppm, significantly more males showed basophilic foci in the liver. At 2000 ppm, animals of both sexes showed reduced cerebral mineralization. This effect might be caused by reduced ageing-related normal arteriosclerotic changes. At the highest dose, males showed increased erythrophagocytosis in the mesenteric lymph node.

An increase in liver adenomas and carcinomas in males at 400 ppm was observed, reaching statistical significance for carcinomas in animals in the groups receiving the lowest and intermediate doses (adenomas, 7/50, 5/50, 10/50 and 5/49; carcinomas, 0/50, 3/50, 4/50 and 2/49, in order of increasing dose). Since there is no dose–response relationship for the incidences of adenoma, the Meeting considered that they were of no concern for humans. Two females at 2000 ppm had luteomas, while there were none in the other groups. Animals in the historical control groups had these tumours at incidences ranging from of 0.9% to 10%. The NOAEL was 400 ppm, equal to 60.2 mg/kg bw per day, on the absis of effects on the liver at 2000 ppm (Schladt, 1998).

Rats

Groups of 60 male and 60 female Bor:WISW rats were fed diets containing triadimenol (purity, 94.9%; A:B = 60:40) at a concentration of 0, 125, 500 or 2000 ppm for 2 years. The average daily intakes of triademenol were 0, 7, 25 and 105 mg/kg bw per day for

Table 4. Histopathological findings in the liver of mice fed diets containing triademenol for 80 weeks

Finding	Dietary concentration (ppm)							
	0	80	400	2000	0	80	400	2000
	Males				Females			
Hepatocellular hypertrophy	5	8	34**	49**	2	2	2	45**
Pigmentation	7	7	10	36**	29	33	25	40**
Single cell necrosis	6	9	20**	42**	8	5	8	25**
Basophilic foci	0	3*	2	5**	0	1	0	2

From Schladt (1998)
* $p = 0.05$
** $p = 0.01$

Table 5. Relative mean weights of organs (mg/100 g) in rats fed diets containing triademenol for 2 years

Dietary concentration (ppm)	Lung	Liver	Spleen	Kidney	Testes or ovaries
Males					
0	436	3795	197	703	923
125	414	3916	306	766	963
500	434	3861	195	715	901
2000	419	3867	187	709	942*
Females					
0	513	3962	271	751	50.9[a]
125	528	3829	231	764	56.0[a]
500	540	4139	244	765	57.0[a]
2000	540*	4781**	239*	811*	75.1**[a]

From Kroetlinger et al. (1982)

[a] Statistical analysis of the raw data was performed by the Meeting, in view of inappropriate values in the statistics presented in the study

* $p = 0.05$

** $p = 0.01$

males and 0, 9, 35 and 144 mg/kg bw per day for females. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

Males and females at 2000 ppm showed reduced body-weight gain throughout the study, while consumption of food was not affected. The relative weights of the spleen and ovaries were reduced, but increases in weights of lungs, liver, kidneys and ovaries in females at 2000 ppm were seen (Table 5). In males at 2000 ppm, relative weights of the testes were increased. Statistically significant observations in the blood of animals treated with triademenol at 2000 ppm were reduced erythrocyte counts for both sexes and reduced haemoglobin and erythrocyte volume fraction for females at 6 months, as well as reduced eosinophilic granulocyte counts in females at 500 and 2000 ppm. Although statistically significant, these findings were mostly within the physiological range. At doses of 2000 ppm, transaminase activities (ALT and AST) were increased by less than twofold in both sexes, and glutamate dehydrogenase activity was increased by nearly three-fold in males. In males at 2000 ppm, reduced protein concentrations were found. In males at ≥125 ppm, lower creatinine values were found, while in females at ≥500 mg/kg, higher urea values in the plasma were found.

There was no histopathological evidence for treatment related non-neoplastic or neoplastic changes.

The NOAEL was 500 ppm, equal to 25 mg/kg bw per day, on the basis of effects on organ weights at 2000 ppm (Kroetlinger et al., 1982).

2.4 Genotoxicity

The results of studies of genotoxicity with triademenol are summarized in Table 6.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a three-generation study, groups of 10 male and 20 female Long Evans FB 30 rats were fed diets containing triadimenol (purity not reported) at a concentration of 0, 125, 500

Table 6. Studies of genotoxicity with triadimenol

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation[a]	*S. typhimurium* TA1535, TA1537, TA100, TA98	4–2500 µg/plate +S9 2500 µg/plate −S9, in DMSO	93.7	Negative Negative	Herbold (1979a)
Reverse mutation[b]	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98 and *E. coli* WP2 hcr	5–5000 µg/plate ±S9, in DMSO	97.5	Negative	Tanahashi & Moriya (1982)
Reverse mutation[b]	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98 and *E. coli* B/r WP2 try$^-$ hcr$^-$	5–5000 µg/plate ±S9, in DMSO	97.5	Negative	Nagane et al. (1982)
Forward mutation[c]	Mouse lymphoma L5178Y *Tk*$^{+/-}$ cells	25–150 µg/ml ±S9	97.5	Negative	Cifone (1982)
Rec assay	*Bacillus subtilis* H17 (rec$^+$) and M45 (rec$^-$)	50–10 000 µg/disk	97.5	Negative	Tanahashi & Moriya (1982)
Rec assay	*B. subtilis* NIG17 (rec$^+$) and NIG45 (rec$^-$)	200 µg/disk	97.5	Negative	Nagane et al. (1982)
Unscheduled DNA synthesis[d]	Primary hepatocytes from male F344 rats	0.25–50 µg/ml	97.5	Negative	Myhr (1982)
DNA damage	*E. coli* (K12)p 3478 (pol A$_1^-$) and W3110 (pol A$^+$)	62.5–1000 µg/plate	97.5	Negative	Herbold (1981)
Sister chromatid exchange	Chinese hamster ovary K1 cells (CHO)	38–300 µg/ml −S9 100–200 µg/ml +S9[e] 125–225 µg/ml +S9	93.0	Negative Positive Negative	Putman (1987)
In vivo					
Micronucleus formation	Bone-marrow erythroblasts of male and female NMRI mice	Two oral doses at 175 or 350 mg/kg bw	93.7	Negative	Herbold (1978a)
Micronucleus formation	Bone marrow erythroblasts of male and female NMRI mice	Two oral doses at 350 or 500 mg/kg bw	96.5	Negative	Herbold (1979b)
Dominant lethal mutation	Male NMRI mice	Single oral dose at 500 mg/kg bw	93.7	Negative	Herbold (1978b)

DMSO, dimethylsulfoxide; S9, 9000 × *g* rat liver supernatant

[a] Bacteriotoxicity observed at doses of > 500 µg/plate.

[b] Bacteriotoxicity observed at doses of > 1000 µg/plate.

[c] Cytotoxicity observed at 250 µg/ml.

[d] Reduced survival (53%) at 50 µg/ml.

[e] The first assay with S9 gave positive results (14.66–15.62 sister chromatid exchanges (SCEs) per cell in treated cells and 12.90 SCEs per cell in non-treated cells) at all doses tested, without dose dependency. The confirmatory assay gave negative results.

or 2000 ppm. Although an old study, it had not been evaluated previously. The pretreatment period before the first mating was 70 days. In each generation, 10 male and 20 female pups of the second of two matings (F_{1b}, F_{2b}, F_{3b}) were used to produce the next generation. All female animals were kept consecutively for a period of longer than one estrus cycle with each of three males. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

The fertility in the F_0 generation decreased at dietary concentrations of ≥125 ppm (statistically significant at 2000 ppm) with fewer pregnant rats and fewer F_1 pups born, and a non dose-dependent increase in the male : female ratio in the F_{1b} litters at dietary concentrations of ≥125 ppm and reduced viability of pups at 2000 ppm (Table 7). The body weights of F_1 pups at birth were not affected. Fertility in the F_1 generation decreased at doses of ≥500 ppm, with fewer pregnant rats and fewer F_2 pups born, and significantly reduced viability of pups was observed in the first mating at doses of ≥125 ppm and in the second mating at 2000 ppm at 4 weeks. Although reduced pup viability was statistically significant also at 125 ppm, the Meeting considered it to be marginal. No changes in the sex ratio were seen in F_2 animals. Reduced body-weight gains at ≥125 ppm and reduced fertility was seen in F_2 animals at dietary concentrations of ≥500 ppm with fewer pregnant rats and fewer F_3

Table 7. Reproductive parameters[a] in a three-generation study in rats fed with diets containing triademenol

Parameter	Dietary concentration (ppm)			
	0	125	500	2000
F_0 1st mating	17/20 (85)	16/20 (80)	14/19 (73.7)	4/20 (20**)
F_0 2nd mating	16/18 (88.9)	16/20 (80)	14/19 (73.7)	13/19 (68.4)
F_1 1st mating	20/20 (100)	20/20 (100)	14/20 (70*)	4/8 (50**)
F_1 2nd mating	16/14 (84.2)	17/20 (85)	6/20 (30**)	4/7 (57.1)
Viable F_{2a} pups at 4 weeks	98.9	93.9*	70.3**	63.6**
Viable F_{2b} pups at 4 weeks	97.3	91.3	95.8	73.3**

From Loeser & Eiben (1982)
[a]The values given are No. of pregnant rats/No. of mated rats (%)
*$p = 0.05$
**$p = 0.01$

pups born. The birth body weights of F_2 pups were decreased only at dietary concentrations of ≥500 ppm in the first mating and at 2000 ppm in the second mating. All pups of the first F_2 mating of animals fed diets containing triademenol at dietary concentrations of ≥500 ppm died within the first 4 weeks. This effect was not seen in the second mating. On gross pathological examination, no malformations in the pups were detected. Histopathology was not performed. Organ weight analyses of all F_{2b} parents revealed significantly increased testes weights at dietary concentrations of ≥500 ppm. In all three generations, body-weight gain was reduced in animals of both sexes, in the F_0 and F_1 at 2000 ppm only and in the F_2 at dietary concentrations of ≥125 ppm.

The NOAEL for maternal toxicity was 2000 ppm, the highest dietary concentration tested. The NOAEL for reproductive toxicity was 125 ppm, equivalent to 12.5 mg/kg bw, on the basis of reduced viability of pups at dietary concentrations of ≥500 ppm (Loeser & Eiben, 1982).

In a two-generation study, groups of 10 male and 20 female Bor:WISW rats were fed diets containing triadimenol (purity, 97.5%; A:B = 80:20) at a concentration of 0, 20, 100 or 500 ppm. The pretreatment period before the first mating was 70 days. In each generation, 10 males and 20 females of the second of two matings (F_{1b}, F_{2b}) were used to produce the next generation. All female animals were kept consecutively for a period of longer than one estrus cycle together with each of three males. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

Males of the F_{1b} generation at all doses had significantly decreased body-weight gains (<10%). The insemination indices (ratio of inseminated:not inseminated female rats) of both F_1 matings were decreased at 500 ppm (70% and 80% versus 95% and 100% in control groups). This effect was not commented on by the study authors. F_{2a} pups of both sexes showed reduced body-weight gains at 500 ppm. Histopathological examination of organs in F_{1b} parents and F_{2b} pups at 0 and 500 ppm revealed no treatment-related changes. Organ weight analysis in F_{1b} parents showed statistically significantly increased relative weights of the testes and ovaries at the highest dietary concentration.

The NOAEL for parental and reproductive toxicity was 100 ppm, equal to 8.6 mg/kg bw per day in F_0 parents, on the basis of reduced body-weight gains, effects on the weights of testes and ovaries, and reduced insemination indices at 500 ppm (Loeser & Eiben, 1984).

(b) Developmental toxicity

Rats

Groups of 20–22 mated female FB 30 rats (Long Evans) were given triadimenol (purity, 93.7%; A:B = 60:40) at a dose of 0, 10, 30 or 100 mg/kg bw per day by gavage from day 6 to day 15 of gestation. On day 20 of gestation, the fetuses were removed by cesarean section. This is a pre-GLP study and no statement of QA was provided.

At 100 mg/kg bw per day, the body-weight gain was slightly reduced and 20 out of 22 inseminated animals (90.9%) were fertilized; in all other groups, all females were fertilized. The study authors considered that the slight reduction at the highest dose was not treatment related. At the highest dose, increased fetal and placental weights were observed.

The NOAEL for maternal and offspring toxicity was 30 mg/kg bw per day on the basis of minor fertility and developmental effects, and placental weight effects at 100 mg/kg bw per day (Machemer, 1977a).

Groups of 25 mated female Wistar/HAN rats were given triadimenol (purity, 97%; A:B = 80:20) at a dose of 0, 30, 60 or 120 mg/kg bw per day by gavage from day 6 to day 15 of gestation. On day 21 of gestation, the fetuses were removed by cesarean section. This study complied with OECD requirements for GLP. At 60 and 120 mg/kg bw per day, body-weight gain and the food consumption were slightly reduced (statistically significantly). Additionally, at the highest dose, an increase in postimplantation loss was observed.

The NOAEL for maternal toxicity was 30 mg/kg bw per day on the basis of body-weight effects at 60 mg/kg bw per day.

The NOAEL for offspring toxicity was 60 mg/kg bw per day on the basis of postimplantation losses at 120 mg/kg bw per day (Becker et al., 1987a).

Groups of 25 mated female Long Evans BAY:FB30 rats were given triadimenol (purity, 95.2%; A:B = 80:20) at a dose of 0, 10 or 30 mg/kg bw per day by gavage from day 6 to day 15 of gestation. On day 20 of gestation, the fetuses were removed by cesarean section. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

At the highest dose, maternal body-weight gain was reduced and placenta weights were increased. The study author stated that this was ". . . a familiar result of treatment with azoles".

The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of body-weight effects at 30 mg/kg bw per day. The NOAEL for offspring toxicity was 10 mg/kg bw per day on the basis of increased placental weights at 30 mg/kg bw per day (Renhof, 1984).

Groups of 28 mated female Charles River Crl:CDBR rats were given triadimenol (purity, 95%) at a dose of 0, 5, 15, 25 or 60 mg/kg bw per day by gavage from day 6 to day 15 of gestation. On day 20 of gestation, the fetuses were removed by cesarean section. This study complied with OECD requirements for GLP.

At doses of ≥15 mg/kg bw per day, body-weight gain was reduced (about 10%) from day 6 to day 16 with a concomitant reduction in feed consumption. Placenta weights were increased at 60 mg/kg bw per day (Table 8). At doses of ≥25 mg/kg bw per day, an increase in the incidence of supernumerary lumbar ribs was observed.

The NOAEL for maternal toxicity was 25 mg/kg bw per day on the basis of body-weight effects at 15 mg/kg bw per day. The NOAEL for offspring toxicity was 15 mg/kg bw per day on the basis of increased supernumerary lumbar ribs at 25 mg/kg bw per day (Clemens et al., 1990b).

Rabbits

Groups of 20 artificially inseminated female New Zealand White rabbits were given triadimenol (purity, 96%) at a dose of 0, 5, 25 or 125 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 29 of gestation, the fetuses were removed by cesarean section. This study complied with OECD requirements for GLP.

At the highest dose, body-weight gain was reduced, as was food consumption. In the groups receiving the intermediate or the highest dose, there was a statistically significant decrease in median litter size (5.2 and 5.6 versus 7.6), implantations per litter (5.7 and 6.3 versus 8.4) and an increase in pre-implantation losses (23.4% and 14.7% versus 10.7%) (Table 9). This corresponded to reduced numbers in corpora lutea graviditatis (7.0 and 7.0 versus 9.0). After comparing these values with the data for historical controls, the Meeting therefore judged that these minimal reproductive effects were not treatment-related. A statistically significant increase in placental weight was observed only at the intermediate dose.

Table 8. Developmental parameters in a study of developmental toxicity in rats given triademenol by gavage

	Dose (mg/kg bw per day)				
	0	5	15	25	60
Body-weight gain days 6–16 (g)	52.5	51.7	45.8*	45.9**	39.9**
Placental weights (g)	0.52	0.52	0.51	0.55	0.63**
Extra ribs	1	6	6	13**	42**

From Clemens et al. (1990b)
*$p = 0.05$
**$p = 0.01$

Table 9. Reproductive efficiency in a study of developmental toxicity in rabbits given triademenol by gavage

Parameter		Dose (mg/kg bw per day)				Range for six historical control groups
		0	5	25	125	
Litter size	Mean	7.6	7.3	5.2	5.6	5.6–8.5
	Median	8.0	7.5	5.0*	6.0*	6–8
Implantations per litter	Mean	8.4	7.5	5.7	6.5	5.6–8.5
	Median	9.0	8.0	6.0**	6.0*	6–9
Pre-implantation losses (%)	Mean	10.7	12.0	23.4	14.7	5.1–23.6
	Median	9.1	0.0	25	6.3	0–11.1

From Clemens et al. (1992)
*$p = 0.05$ using Dunn test
**$p = 0.01$ using Dunn test

The NOAEL for maternal toxicity was 125 mg/kg bw per day on the basis of body-weight effects at 125 mg/kg bw per day. The NOAEL for offspring toxicity was 125 mg/kg bw per day, the highest dose tested (Clemens et al., 1992).

Groups of 16 mated female Chinchilla rabbits were given triadimenol (purity, 97%; A : B = 80 : 20) at a dose of 0, 8, 40 or 200 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 28 of gestation, the fetuses were removed by cesarean section. This study complied with GLP (unknown authority) requirements.

Animals at the highest dose showed excited behaviour and hair loss on paws and chest, probably caused by gnawing and scratching. At ≥40 mg/kg bw per day, the body-weight gain was reduced (<10%), concomitantly with reduced food intake at the highest dose. At the highest dose, a slight increase of postimplantation losses was observed. No other treatment-related effects on reproduction and development were observed.

The NOAEL for maternal toxicity was 40 mg/kg bw per day on the basis of reduced body-weight gain at ≥40 mg/kg bw per day. The NOAEL for offspring toxicity was 40 mg/kg bw per day on the basis of slightly increased postimplantation losses at 200 mg/kg bw per day (Becker et al., 1987b).

2.6 Special studies: neurotoxicity

In a study of neurotoxicity, male mice (Bor : CFW1) and male rats (Bor : WISW) were given single doses of triadimenol (purity, 98%) by gavage. In mice, effects on hexobarbital sleeping time, spontaneous motility, behaviour, open field behaviour and reserpine-induced ptosis were examined, while in rats, behaviour in a "novel box" test was investigated.

In mice, doses of 3.75 to 60 mg/kg bw per day stimulated the spontaneous motility, increased the irritability escape response and certain reflexes; doses of 15 and 60 mg/kg bw per day intensified the effect of amphetamine; and doses of 12–48 mg/kg bw per day antagonized that of reserpine. The test substance prolonged hexobarbital sleeping time at 15 and 60 mg/kg bw per day.

In rats, triademenol at 48 mg/kg bw per day had an excitatory effect and therefore reduced the sleeping time.

A comparison with caffeine showed that behavioural effects of 2.5 mg of caffeine/kg bw corresponded approximately to that of 12–15 mg of triadimenol/kg, in the amphetamine potentiation test 2.5 mg of caffeine/kg bw compared with 4 mg of triadimenol/kg and in the antagonism of ptosis, 10 mg of caffeine/kg bw and 12 mg of triadimenol/kg had similar potency (Polacek, 1983a). Owing to small group sizes, uncertain significance of the end-points and the lack of standardized study protocols, the Meeting concluded that this study would not be considered for evaluation of a reference dose.

In a comparative study to reveal structure–activity relationships with respect to neurological effects, groups of 8–12 male Long Evans rats were given a single dose of one of 14 different triazole fungicides or structurally related compounds by gavage. Dose ranges for the individual compounds were selected mainly according to their acute toxicity. The animals were subjected to the figure-eight maze test. Behavioural changes expressed as increased activity were restricted to triadimenol and triadimefon, reaching statistical

significance at 100 mg/kg bw per day. In both cases, a tendency to increased activity was observed at the lowest dose tested, 50 mg/kg bw per day (Crofton, 1996).

In studies in vitro, it was shown that triadimenol and triadimefon had a significant dopamine transporter-binding capacity, with no dopamine-releasing function in the striatum but with a dopamine uptake-inhibiting effect in striatal synaptosomal preparations (Walker & Mailman, 1996; Ikaiddi et al., 1997).

3. Observations in humans

In a medical survey spanning more than 10 years, no substance-related effects were observed in persons producing and formulating triadimenol. However, it was stated that no evidence of exposure had been found (Kehrig, 1999).

TRIADIMEFON

Evaluation for acceptable daily intake: triadimefon

4. Biochemical aspects

4.1 Absorption, distribution, excretion and metabolism

Five male and five female Wistar rats were given [phenyl-UL-^{14}C]triadimefon (specific activity, 15.78 mCi [583.9 MBq]/mmol) at a dose of 5 mg/kg bw or 50 mg/kg bw by gavage. In a study of multiple doses, 10 animals of each sex were treated with unlabelled triadimefon at a dose of 5 mg/kg bw per day for 14 days and on day 15 with [phenyl-UL-^{14}C]triadimefon at a dose of 5 mg/kg bw. In all these studies, animals were terminated 96 h after treatment with the radiolabelled substance. Expired air, faeces and urine were collected for analyses of radioactivity and metabolite. This study complied with the requirements of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA) for GLP.

The pattern of excretion and metabolism of the radiolabel was not significantly affected at either dose, or by pretreatment with unlabelled triadimefon. In males, 24–28% and in females, 57–67% of the administered dose was excreted in the urine, and 63–66% and 32–41% was excreted in the faeces, respectively. Less than 1% of the administered dose was expired. After 96 h, 2% of the radiolabel remained in the bodies of females and 9% in males, with the highest residue concentrations in the liver and kidneys.

The metabolism of triadimefon starts either by direct oxidation of the *t*-butyl methyl group to the hydroxy or the carboxy compound, with subsequent glucuronidation, or these steps are preceded by reduction of the keto group to the putative intermediate triadimenol. Therefore many of the metabolites found in studies of the metabolism of triadimenol (Puhl & Hurley, 1978; Justus, 2002b) are also found with triadimefon. Nevertheless, the metabolism of triadimefon in rats provides a pathway of demethylation of the *t*-butyl group that is not seen, at least not to any significant degree, with triadimenol (Chopade, 1992).

A single lactating goat aged 2 years was treated orally with capsules containing [phenyl-UL-^{14}C]triadimefon at a dose of 2.59 mg/kg bw per day (specific activity, 38.5 mCi [1.4 GBq]/mmol) for 3 consecutive days. On the third day, the animal, milk, faeces, urine and organs were collected for radioactivity analyses. This study complied with FIFRA requirements for GLP.

Within 24 h, 83% of the radioactivity was excreted in the urine. Highest residue concentrations were found in the kidneys (3.5 mg/kg) and in the liver (1.6 mg/kg). The residue concentration found in muscle was 0.068 mg/kg. Residue concentrations found in the milk at 0–24 h and at 24–48 h were similar (0.029 mg/kg) (Hall & Hartz, 1993).

Qualitatively, the metabolic pattern of triadimefon in the goat closely resembled that in rats (Chopade, 1992).

Ten laying hens were treated orally with capsules containing [phenyl-UL-^{14}C]triadimefon (specific activity, 38.5 mCi [1.4 GBq]/mmol) at a dose of 2.45 mg/kg bw per day for 3 consecutive days. On the third day, liver, fat and muscle were collected for radioactivity analyses. This study complied with FIFRA requirements for GLP.

Concentrations of radioactive residue in eggs increased from 0.007 (day 1) to 0.088 mg/kg (day 3). In the liver, fat and muscle, residue concentrations of 0.731, 0.171 and 0.123 mg/kg, respectively, were found (Duah & Smasal, 1993).

Qualitatively, the metabolic pattern of triadimefon in hens closely resembled that in rats (Chopade, 1992).

4.2 Effects on enzymes and other biochemical parameters

In a set of experiments in vitro and in vivo, the interaction of triadimefon with liver microsomal enzymes from mice and rats was investigated. No statement of compliance with GLP was provided.

Spectral analyses showed binding of triadimefon to cytochrome P450 in vitro and inhibition of monooxygenase activity was observed.

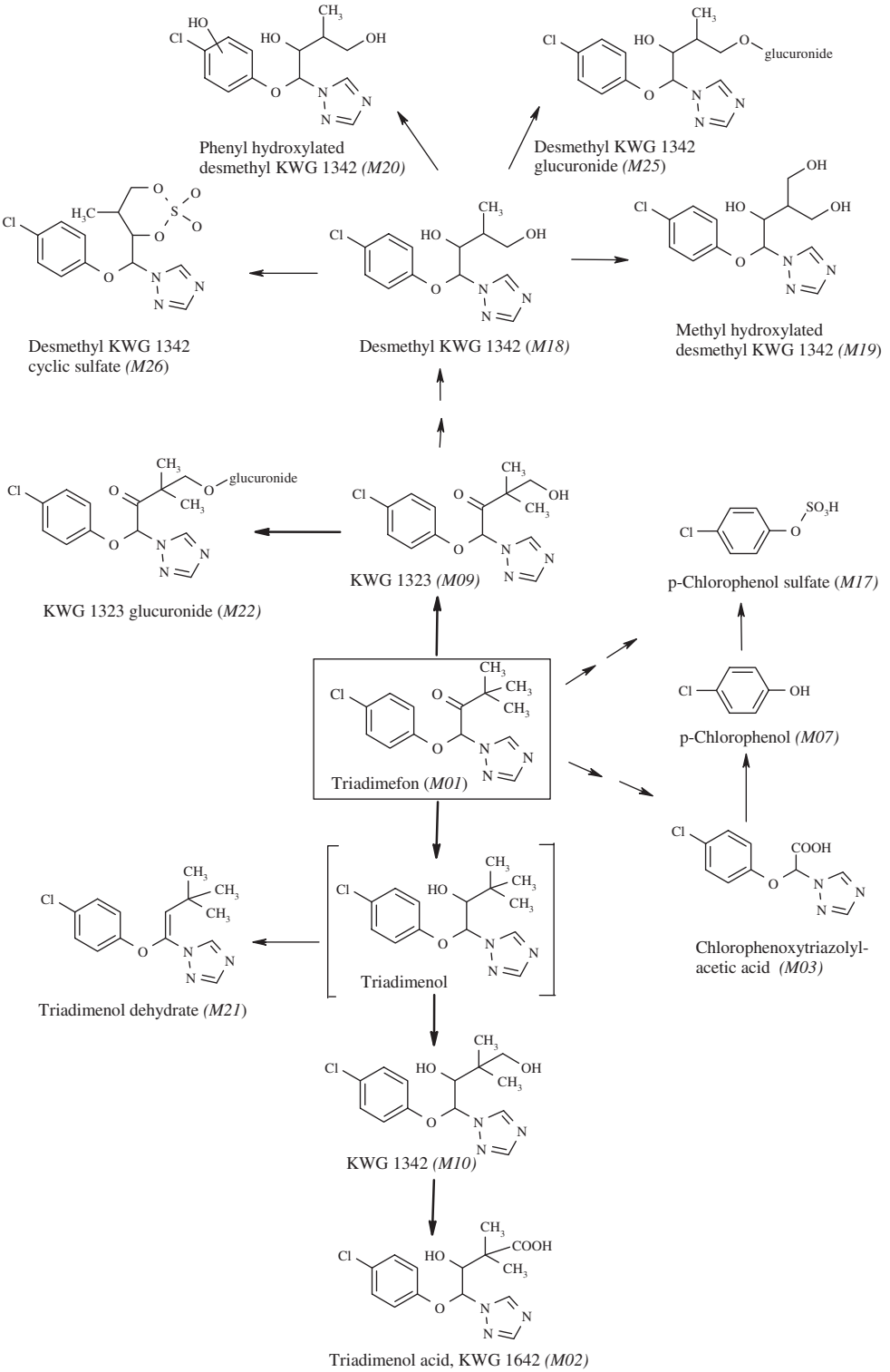
On exposure to triadimefon, induction of monooxygenase activity was observed, which was slight in rats and more pronounced in mice.

On the basis of the absence of any change in biphenyl 2-hydroxylase activity and the induction of aldrin epoxidation, it was concluded that enzyme induction by triadimefon more closely resembles that by phenobarbital than that by ligands of the aryl hydrocarbon (Ah) receptor (Schmidt, 1983). This is consistent with other azoles, e.g. propiconazole.

In a comparative assay for enzyme inhibition in vitro, the effects of triadimenol and triadimefon and other pesticides on aromatase activity were studied. Aromatase converts androgens to estrogens and therefore is important for achieving a balance in levels of sex steroids. Half maximal inhibition of human placental microsomal aromatase was observed for triadimenol and triadimefon at 21 µmol/l and 32 µmol/l, respectively. This was judged to be a weak inhibition when compared with that attributed to other azole compounds, such as prochloraz (Vinggaard et al., 2000).

Both compounds were found to be weak estrogen receptor agonists in MCF7 breast cancer cells (triadimefon and triadimenol at 10 µmol/l induced a 2.4-fold and 1.9-fold increase in cell proliferation respectively), but not in estrogen receptor alpha-transfected yeast cells. Other azole compounds such as prochloraz and imazalil either gave negative results in these assays or had very low activity (Vinggaard et al., 1999).

Figure 2. Proposed metabolism[a] of triadimefon in rats



From Bayer CropScience AG (2003)

[a]The scheme was reproduced from the manufacturer's evaluation

5. Toxicological studies

5.1 Acute toxicity

The acute toxicity of tridimefon is summarized in Table 10.

(a) Dermal and ocular irritation

In New Zealand White rabbits, slight treatment-related dermal and ocular irritation reactions were observed in a few cases (Sheets, 1990b; Sheets, 1990c).

(b) Dermal sensitization

Triadimefon (purity, 94.6%) was sensitizing to the skin of guinea-pigs in the Buehler topical test (Sheets, 1990a).

In the Magnusson-Kligman maximization test, technical-grade triadimefon (purity 94.6%) was used for intradermal and topical induction. Challenge with technical-grade triadimefon clearly resulted in sensitization, while challenge with purified triadimefon (purity, 99.6%) gave negative results (Diesing, 1991).

Table 10. Acute toxicity of triadimefon

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	LC_{50} (mg/l)	Reference
Mouse	NMRI	Male	Oral (fasted)	92.6	732	—	Mihail (1980)
Mouse	NMRI	Female	Oral (fasted)		1158	—	
Mouse	NMRI	Male	Oral	93.4	989	—	Thyssen & Kimmerle (1974)
Mouse	NMRI	Female	Oral		1071	—	
Mouse	NMRI	Male	Inhalation		—	>0.516	
Rat	Wistar	Male	Oral (fasted)	92.6	1855	—	Mihail (1980)
Rat	Wistar	Female	Oral (fasted)		1020	—	
Rat	Wistar	Male	Oral (fed)	93.4	568–1245	—	Thyssen & Kimmerle (1974); Mihail (1980)
Rat	Wistar	Female	Oral (fed)	92.6 and 93.4	363–793	—	Thyssen & Kimmerle (1974); Mihail (1980)
Rat	Wistar	Males and females	Dermal	92.6	>5000	—	Mihail (1980)
Rat	Wistar	Males and females	Intraperitoneal	92.6 and 93.4	213–321	—	Thyssen & Kimmerle (1974); Mihail (1980)
Rat	Wistar	Male	Dermal	93.4	>1000	—	Thyssen & Kimmerle (1974)
Rat	Wistar	Males and females	Inhalation		—	>0.455	
Rat	Sprague-Dawley	Male/Female	Inhalation	95.0	—	>3.27	Warren (1990)
Hamster		Male	Inhalation	93.4	—	>0.516	Thyssen & Kimmerle (1974)
Rabbit	NZW	Female	Oral		500	—	Thyssen & Kimmerle (1974)
Rabbit	NZW	Male	Oral (fasted)	92.6	250–500	—	Mihail (1980)
Hen		Female	Oral	93.4	500	—	Thyssen & Kimmerle (1974)
Quail		Female	Oral		1750–2500	—	Thyssen & Kimmerle (1974)

NZW, New Zealand White

5.2 Short-term studies of toxicity

Rats

Groups of 15 male and 15 female Wistar rats were given triadimefon (purity, "technical grade") at a dose of 0, 3.0, 10.0 or 30.0 mg/kg bw per day by gavage for 30 days. This is a pre-GLP study and no statement of QA was provided.

There were no treatment-related findings on behaviour, body-weight development, haematology, clinical chemistry, urine analysis or histopathology. The only treatment-related findings were increased relative and absolute weights of the liver in males at dosesof ≥10 mg/kg bw per day and in females at 30 mg/kg bw per day. There were no histopathological or clinical chemistry findings indicative of liver damage.

The NOAEL was 30.0 mg/kg bw per day, the highest dose tested (Thyssen et al., 1974).

Groups of 20 male and 20 female Wistar rats were given triadimefon (purity, 97%) at a dose of 0, 1, 5 or 25 mg/kg bw per day by gavage for 4 weeks, followed by a 4-week recovery period. This is a pre-GLP study and no statement of QA was provided.

At doses of ≥5 mg/kg bw per day, mild induction of microsomal enzymes was observed. This effect was reversible in the recovery period. No other parameters (appearance, behaviour, body weight, haematology, clinical chemistry, gross pathology, liver weight or histopathology) were affected.

The NOAEL was 25 mg/kg bw per day, the highest dose tested (Mihail & Kaliner, 1979).

Groups of 15 male and 15 female Sprague-Dawley rats were fed diets containing triadimefon (purity not reported) at a concentration of 0, 50, 200, 800 or 2000 ppm for 12 weeks. This is a pre-GLP study and no statement of QA was provided.

There were no treatment-related changes in appearance, behaviour, body weight, haematology, clinical chemistry, urine analysis, gross pathology or histopathology.

The NOAEL was 2000 ppm, equal to 150 mg/kg bw per day, the highest dose tested (Mohr, 1976).

Groups of five male and five female Sprague-Dawley rats were treated dermally with triadimefon (purity, 95.9%) at a dose of 0, 100, 300 or 1000 mg/kg bw per day for 6 h per day, 5 days per week, over a period of 3 weeks. This study complied with OECD requirements for GLP.

Females at the highest dose showed increased activity and reactivity as well as an increased incidence of diffuse acanthosis at the application site. No other parameters, including appearance, behaviour, body and organ weights, haematology, clinical chemistry, gross pathology, urine analysis or histopathology, were affected.

The NOAEL was 300 mg/kg bw per day on the basis of behavioural effects at 1000 mg/kg bw per day (Sheets & Phillips, 1992).

In the first of two studies of exposure by inhalation, groups of 10 male and 10 female Wistar rats were given triadimefon at a concentration of 0.454 mg/l by daily inhalation for 4 h on 5 consecutive days. The post-exposure period was 14 days (Thyssen et al., 1974).

In the second study, groups of 10 male and 10 female Wistar rats were given triadimefon at a concentration of 0.079 or 0.307 mg/l by daily inhalation for 6 h on 15 days (3×5 consecutive days over 3 weeks). After the last exposure, the animals were terminated. This is a pre-GLP study and no statement of QA was provided.

The only treatment-related finding was reduced body-weight gain in males at the higher dose in the second study and increased relative liver weights in males and females at the higher dose in the same study.

The NOAEC was 0.079 mg/l (Thyssen et al., 1974).

Rabbits

Groups of 15 mated female American Dutch rabbits were given triadimefon (purity, 94.7%) at a dose of 0, 20, 50 or 120 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 19 of gestation, the dams were terminated. No examinations on reproductive and developmental effects were performed. This study complied with OECD requirements for GLP.

At the highest dose, reduced food consumption, a loss in body weight and increased spleen and adrenal weights were observed. In the spleens, increased incidences of reticuloendothelial cell hyperplasia and macrophages with cell debris were found.

The NOAEL was 50 mg/kg bw per day on the basis of body weight and organ weight changes with histopathological correlates at 120 mg/kg bw per day (Clemens et al., 1990a).

Groups of three male and three female rabbits were treated dermally (intact or abraded skin) with triadimefon (purity, "pure technical grade") at a dose of 0, 50 or 250 mg/kg bw per day on 5 days per week for 4 weeks. This is a pre-GLP study and no statement of QA was provided.

The only treatment-related finding was slight erythema in all dosed animals (intact and abraded skin). No other parameters including appearance, behaviour, body and organ weights, haematology, clinical chemistry, gross pathology, urine analysis or histopathology were affected.

The NOAEL was 250 mg/kg bw per day, the highest dose tested (Thyssen & Weischer, 1976).

Dogs

Groups of four male and four female beagle dogs were fed diets containing triadimefon (purity, 99.6%) at a concentration of 0, 150, 600 or 2400 ppm for 13 weeks. This is a pre-GLP study and no statement of QA was provided. The average daily intakes of triadimefon were 0, 4.4, 17.3 and 65.8 mg/kg bw per day (calculated from the daily intakes per animal of 0, 43.5, 173 and 658 mg and a default body weight of 10 kg).

Table 11. Findings in dogs fed with triademefon for 2 years

	Dietary concentration (ppm)			
	0	100	300	1000–2000
Mean body weight at the end of study (kg)	10.86	11.74	10.76	10.05
N-Demethylase (nmoles/g per min)	35.8	40.5	43.4	99.6
AST (U/l)	20.4	20.8	23.7	19.0
aLT (U/l)	27.8	25.8	26.5	26.6
ALP (U/l)	113.6	114.5	133.0	565.63

From Hoffmann & Groening (1978)
ALP, alkaline phosphatase; ALT, alanine transaminase; AST, aspartate aminotransferase

Clinical inspections, haematology, clinical chemistry, urine analysis, gross and histological pathology were performed. Body-weight gain, food intake, relative weights of the liver and general appearance of animals at the highest dose were impaired. In this group, erythrocyte volume fraction, erythrocyte counts and haemoglobin were reduced. At doses of ≥600 ppm, animals showed an increased aminopyrine-*N*-demethylating activity, and at 2400 ppm, increased plasma ALP and ALT activity.

The NOAEL was 600 ppm, equal to 17.3 mg/kg bw per day, on the basis of a number of effects at 2400 ppm (Hoffmann & Luckhaus, 1974).

Groups of four male and four female beagle dogs were fed diets containing triadimefon (purity, 88.9%) at a concentration of 0, 100, 330 and 1000 ppm for 2 years. The average daily intakes of triademefon were 0, 3.26, 11.7 and 48.8 mg/kg bw per day. This study did not comply with GLP requirements, but was supervised by an internal QA unit. The dose of the group at 1000 ppm was increased to 2000 ppm from week 55 to 104.

At the highest dose, a slight significant decrease in body-weight gain and mild induction of hepatic microsomal enzymes was observed (Table 11). There were no other treatment-related findings.

The NOAEL was 2000 ppm, equal to 48.8 mg/kg bw per day, the highest dose tested (Hoffmann & Groening, 1978).

5.3 Long-term studies of toxicity and carcinogenicity

Mice

Groups of 60 male and 60 female NMRI mice were fed diets containing triadimefon (purity not reported) at a concentration of 0, 50, 300 or 1800 ppm for 21 months. The average daily intakes were 0, 13.5, 76 and 550 mg/kg bw per day for males and 0, 19.6, 119 and 765 mg/kg bw per day for females. Ten animals per group were terminated at 12 months. This study complied with FIFRA requirements for GLP.

Males at the highest dose showed reduced body-weight gain (approximately 20%). In all treated females, reduced erythrocyte volume fraction and increased mean corpuscular haemoglobin concentration were found without any clear dose dependence. Increased activity of ALT and AST was observed at the highest dose at the end of the study in both sexes, and also in females at the intermediate dose at the interim kill. ALP activity was increased in males at the highest dose. In males at doses of ≥300 ppm, the number of animals with

nodular changes in the liver was increased and in both sexes at 1800 ppm, nodular changes, fatty droplets, discolouration and enlargement of the liver were found. These findings were accompanied by increases in absolute and relative weights of the liver. All dosed females and males at ≥300 ppm showed hepatocellular hypertrophy and an increase in single cell necrosis. Females at ≥300 ppm and males at 1800 ppm showed increased Kupffer cell proliferation, multifocal round cell infiltrations and lipofuscin deposits in macrophages. In males and females at the highest dose, an increase in hepatocellular adenomas was observed relative to that in controls (11 versus 3, and 9 versus 2) (Table 12).

The NOAEL was 50 ppm, equal to 13.5 mg/kg bw per day, on the basis of signs of liver damage at ≥300 ppm (Bomhard & Hahnemann, 1986).

Groups of 50 male and 50 female CF_1/W 74 mice were fed diets containing triadimefon (purity, 97%) at a concentration of 0, 50, 300 and 1800 ppm for 24 months. The average daily intakes of triademefon were 0, 9.29, 60.5 and 421 mg/kg bw per day for males and 0, 12.0, 75.6 and 471 mg/kg bw per day for females. This is a pre-GLP study and no statement of QA was provided.

Animals of both sexes at the highest dose showed reduced body-weight gain (<10%) and increased liver weights; the livers appeared swollen, and hardened or brittle (Table 13). Males in this group also had reduced kidney weights. All dosed males showed reduced mean corpuscular haemoglobin concentrations. At the end of the study, both sexes in the group receiving the highest dose had increased erythrocyte counts and females had increased thrombocyte counts and increased haemoglobin, erythrocyte volume fraction and mean corpuscular haemoglobin concentrations. At the highest dose, animals of both sexes had increased activity of ALP, AST and ALT.

Table 12. Incidence of liver adenomas in mice fed with triademefon for 21 months

	Dietary concentration (ppm)			
	0	50	300	1800
No. of animals of each sex examined	50	50	50	50
Males	3	3	4	11
Females	2	1	0	9

From Bomhard & Hahnemann (1986)

Table 13. Findings at the end of a 24-month study in mice fed diets containing triademefon

Finding	Dietary concentration (ppm)			
	0	50	300	1800
No. of animals examined of each sex	50	50	50	50
Liver weight in males (mg)	2241	2233	2605	4176**
Liver weight in females (mg)	1833	2021	1950	3819**
Kidney weight in males (mg)	853	750*	831	686**
ALP in males (U/l)	151	113	221	1845
ALP in females (U/l)	330	322	291	1135

From Bomhard & Loeser (1980)
ALP, alkaline phosphatase
* $p = 0.05$
** $p = 0.01$

An increase of hyperplastic liver nodules was reported at the highest dose compared with controls (males, 15 versus 7; and females, 15 versus 4). Re-examination of the relevant slides of liver sections 10 years later resulted in reclassification of most of these nodules as liver adenomas and carcinomas. Since not all slides could be re-examined, no final conclusions could be made by the re-examination group regarding putatively increased incidences of neoplastic lesions and their classification.

The NOAEL was 300 ppm, equal to 60.5 mg/kg bw per day, on the basis of body-weight effects and changes in the blood profile (Bomhard & Loeser, 1980).

Rats

Groups of 60 male and 60 female Wistar (Bor : WISW) rats were fed diets containing triadimefon (purity, 94.4%) at a concentration of 0, 50, 300 or 1800 ppm for 105 weeks. Ten animals per group were terminated at 52 weeks. Average daily intakes of triademefon were 0, 2.7, 16.4 and 114 mg/kg bw per day for males and 0, 3.6, 22.5 and 199 mg/kg bw per day for females. This study complied with OECD requirements for GLP.

In spite of an increased food intake, body-weight gain was reduced in both sexes at the highest dose (5–10%) and females showed reduced haemoglobin, mean corpuscular haemoglobin concentration, erythrocyte volume fraction, and reduced erythrocyte counts. Decreased leukocyte counts were observed at different time-points, but there was no apparent dose dependence. In females receiving triademefon at doses of ≥300 ppm and in males at 1800 ppm, absolute and relative weights of the liver were increased. An increase (<50%) in the ALT activity was found in males at 1800 ppm and a decreased AST activity in all dosed females (twofold at the highest dose). In both sexes, a tendency to lower plasma bilirubin values at the intermediate and the highest doses and decreased creatinine values at the highest doses were observed. The males at the highest dose excreted significantly less protein in the urine. In all dosed groups, the incidence of fatty deposits in hepatocyte cytoplasm increased with dose. A marginally-increased incidence of thyroid cystic hyperplasia was found in both sexes at the highest dose, predominantly in females, and concomitantly, a minor increase in the incidence of thyroid follicular adenomas was found compared with controls (males, 3 versus 0; and females, 2 versus 0) (Table 14). A marked decrease in the incidence of several tumours (adrenals in males and mammary glands in females)was observed at the highest dose. In an addendum to the study, the incidences of thyroid adenomas were compared with those for historical controls of this rat strain; they fell well within the ranges for historical controls.

Table 14. Histopathological findings in the thyroid of rats fed diets containing triademefon for 105 weeks

Finding		Dietary concentration (ppm)				Range for historical controls
		0	50	300	1800	
Follicular cell adenomas	Males	0	0	1	3*	0–5.2%
	Females	0	1	0	2	0–4.0%
Cystic hyperplasia	Males	2	3	1	3	—
	Females	2	0	1	4	—

From Bomhard & Schilde (1991)
* $p = 0.05$ in the Peto et al. trend test

The NOAEL was 300 ppm, equal to 16.4 mg/kg bw per day, primarily on the basis of non-neoplastic changes in the thyroid at 1800 ppm (Bomhard & Schilde, 1991).

Groups of 50 male and 50 female Wistar rats were fed diets containing triadimefon (purity, "technical grade") at a concentration of 0 (100 animals of each sex), 50, 500 or 5000 ppm for 24 months. The average daily intakes of triademefon were 0, 2.38 and 24.3 mg/kg bw per day for males and 0, 3.19 and 33.3 mg/kg bw per day for females. No average intake was given for animals in the group at 5000 ppm, since no animals survived the first year of exposure. This is a pre-GLP study and no statement of QA was provided.

Starting on week 23, animals at the highest dose showed violent motor activity, feed refusal and many animals died. After severe body-weight loss, the surviving animals started to feed again but in the following weeks they showed the same symptoms again. On week 39, the last animals at the highest dose were terminated in a moribund state. None of the animals in the groups receiving triademefon at the lowest and the intermediate doses showed any effects on appearance or behaviour. Females at 500 ppm showed slightly reduced body-weight gain. The liver weights of males at ≥50 ppm and the liver and ovary weights of females at 500 ppm were increased (Table 15). There were no histopathological changes in the liver and no clinical chemistry effects indicating liver damage. Adrenal weights of both sexes were decreased at 500 ppm. Animals at the highest dose that died or were killed in extremis showed haemorrhagic lesions in the stomach mucosa, blood-filled and dilated alveolar vessels, degenerative processes in proximal kidney tubules of females, atrophied spleens with signs of decreased haematopoesis, some giant spermatids in testes, and decreased haematopoesis in the bone marrow of males. At doses of ≥500 ppm, females showed statistically significantly reduced and males statistically significantly increased erythrocyte counts (both about 10%). Additionally, males receiving triademefon at doses of ≥500 ppm had statistically significantly (about 70% of that of controls) reduced leukocyte counts. There were no significant enzyme changes indicative of liver damage. In the first 6 months, females at the highest dose showed increased cholesterol concentrations. There were no apparent changes in tumour incidences in any group.

The NOAEL was 50 ppm, equal to 2.38 mg/kg bw per day, on the basis of minimal organ weight changes and changed erythrocyte counts in both sexes at 500 ppm (Bomhard & Loeser, 1978).

Interpolating these two studies, the overall NOAEL was 16.4 mg/kg bw per day.

Table 15. Absolute organ weights (mg) of mice fed with triademefon for 24 months

Organ	Sex	Dietary concentration (ppm)		
		0	50	500
Liver	Males	13 209	15 279**	15 373**
	Females	9 237	9 328	10 143**
Adrenals	Males	46	45	42**
	Females	68	66	60*
Ovaries	Females	129	122	155**

From Bomhard & Loeser (1978)
* $p = 0.05$ in the Wilcoxon-Mann-Whitney U-test
** $p = 0.01$ in the Wilcoxon-Mann-Whitney U-test

Table 16. Studies of genotoxicity with triadimefon

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98	100–3333 µg/plate ±S9, in DMSO	93.1–94.2	Negative	San & Springfield (1990)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA100, TA98	0.1–1000 µg/plate ±S9, in DMSO	97.0	Negative	Inukai & Iyatomi (1977)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98, TA1950	0.1–1000 µg/plate ± mouse microsomes, in DMSO	97.0	Negative	van Dijck (1976)
Reverse mutation[b]	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98, TA1950 *E. coli* WP2 hcr	10–5000 µg/plate in ±S9, DMSO	97.0	Negative	Shirasu et al. (1978); Shirasu et al. (1979)
Reverse mutation	*Saccharomyces* S138 and S211c	0.01–10 µg/plate ±S9, in DMSO	Not reported	Negative	Jagannath (1980)
DNA damage	*E. coli* (K12)p 3478 (pol A_1^-) and W3110 (pol A^+)	625–10 000 µg/plate ±S9	86.0	Negative	Herbold (1984)
Rec assay	*B. subtilis* NIG17 (rec^+) and NIG45 (rec^-)	3–300 µg/disk	97.0	Negative	Inukai & Iyatomi (1977)
Rec assay	*B. subtilis* NIG17 (rec^+) and NIG45 (rec^-)	20–2000 µg/disk	97.0	Negative	Shirasu et al. (1978); Shirasu et al. (1979)
Cytogenetic changes[a]	Primary human lymphocytes	50–200 µg/ml ±S9	93.0	Negative	Herbold (1986)
CHO/HGPRT mutation[a]	Chinese hamster ovary K1 cells (CHO)	105–256 µg/ml ±S9	93.1	Negative	Harbell (1989)
In vivo					
Micronucleus formation	Bone marrow erythroblasts of male and female NMRI mice	Two oral doses at 200 mg/kg bw per day	Not reported	Negative	Machemer (1977b)
Dominant lethal mutation	Male mice	Single oral dose at 200 mg/kg bw	95.9	Negative	Machemer (1976c)
Unscheduled DNA synthesis	Primary rat hepatocytes from male Sprague-Dawley rats	5–160 µg/ml	96.4	Negative	Brendler (1991)

DMSO, dimethylsulfoxide; S9, 9000 × *g* rat liver supernatant

[a] Dose-related cytotoxicity observed at all doses

[b] Shirasu et al. (1978) used only the strains TA98 and TA100.

5.4 Genotoxicity

The results of studies of genotoxicity with triademefon are summarized in Table 16.

5.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a three-generation study, groups of 10 male and 20 female Wistar rats were fed diets containing triadimefon (purity, "technical grade") at a concentration of 0, 50, 300 or 1800 ppm. The pretreatment period before the first mating was 70 days. In each generation, the pups of the second of two matings (F_{1b}, F_{2b}, F_{3b}) were used to produce the next generation. All females were kept for longer than one estrus cycle consecutively with each of three males. This is a pre-GLP study and no statement of QA was provided.

Female and pup body-weight gain was reduced at ≥300 ppm in all generations, reaching statistical significance only at 1800 ppm. At 1800 ppm, fewer (85%) animals became pregnant in the second mating of the F_0. In the first mating of the $F_{1b,}$ only one female became pregnant, while in the second mating none of the females became pregnant. The

sizes of delivered litters at the highest dose were decreased and pup weight survival rate in the lactating period and body-weight gain were impaired. Histopathological examination of the F_{3b} pups revealed no treatment-related effects and at all doses no treatment-related malformations were seen.

The NOAEL for maternal toxicity was 300 ppm, equivalent to 22.8 mg/kg bw per day, on the basis of reduced body-weight gain at 300 ppm. The NOAEL for reproductive toxicity was 300 ppm, equivalent to 22.8 mg/kg bw per day, on the basis of reduced pup weight gain at 1800 ppm (Loeser, 1979).

The above study was supplemented by another study of reproductive toxicity in Bor:WISW rats. Groups of 10 males and 20 females were fed diets containing triadimefon (purity, 92.6%) at a concentration of 0, 50 or 1800 ppm. The pretreatment period before mating was 100 days. In each generation, only one mating was conducted to produce the next generation. All female animals were kept consecutively with each of three males for a week. There was no report on compliance of this study with any GLP standards and no statement on quality assurance was given.

The fertility of the F_0 animals at 1800 ppm was not affected, but the viability (79% versus 93 in controls) and birth weights of the F_1 pups were reduced. The fertility of F_1 animals at 1800 ppm was 35% versus 85% in the control group and the insemination index was 50% versus 100%. Therefore, the ratio of pregnant:inseminated females was 70% at 1800 ppm and 85% in the control group. The litter size, viability and birth weights of the F_2 pups were reduced and the male:female ratio was 38:62, compared with 50:50 in the control group. In a cross mating test, F_1 males at 1800 ppm were mated with F_1 control females (test 1) and F_1 control males were mated with F_1 females of the group at 1800 ppm (test 2). In test 1, the fertility index was 47.4% and in test 2 it was 80%; the respective insemination indices were 63% and 100%, respectively. Therefore, the difference in fertility index probably arose owing to reduced mating willingness in the males at the highest dose. However, the litter size in test 2 was reduced compared to that in test 1 and the male:female ratio was 62:38 (i.e. inverted when compared with the sex ratio observed in the F_2 generation).

In males at the highest dose, the testosterone concentration was double that in control males and testes weights were increased. No correlation between individual testosterone concentrations and spermiograms and mating willingness was observable. Reduced mating willingness appeared to correlate with reduced body weight. In agreement with the study authors, the Meeting concluded that prenatal, but not postnatal exposure of males affects mating willingness.

The NOAEL for reproductive toxicity was 50 ppm, equivalent to 3.75 mg/kg bw per day, on the basis of impaired reproductive performance at 1800 ppm (Eiben, 1984).

(b) Developmental toxicity

Rats

Groups of 26 mated female CD-SD rats were given triadimefon (purity, 93.2%) at a dose of 0, 10, 30 or 90 mg/kg bw per day by gavage from day 6 to day 15 of gestation. On day 20 of gestation, the fetuses were removed by caesarian section. This study complied with FIFRA requirements for GLP.

Table 17. Supernumerary ribs in a study of developmental toxicity in rats treated with triademefon by gavage

Dose (mg/kg bw per day)	Litters affected/total litters	Fetuses affected/total fetuses
0	1/20	1/48
10	10/17	16/52
30	10/19	13/54
90	19/22	57/84

From Unger et al. (1982)

At the highest dose, the body-weight gain of the dams was statistically significantly reduced (29 g compared with 38 g in the controls, only significant from day 6 to day 15 of gestation), and in fetuses, an increase in supernumerary ribs was found (Table 17). No other signs of developmental toxicity were observed. The NOAEL for parental and offspring toxicity was 30 mg/kg bw per day, on the basis of supernumerary ribs in fetuses and body weight depression in dams at 90 mg/kg bw per day (Unger et al., 1982).

Groups of 22–24 mated female FB 30 (Long Evans) rats were given triadimefon (purity not reported) at a dose of 0, 10, 30 or 100 mg/kg bw per day by gavage in test 1 and at a dose of 0, 50, 75 or 100 mg/kg bw per day in test 2, from day 6 to day 15 of gestation. On day 20 of gestation, the fetuses were removed by caesarian section. This is a pre-GLP study and no statement of QA was provided. At doses of ≥30 mg/kg bw per day, the body-weight gain of dams was reduced by ≤50% at 100 mg/kg bw per day. At the highest dose in test 2, there was a slight increase in placental weights.

At 75 mg/kg bw per day and in the two groups at 100 mg/kg bw per day, 2 out of 220 pups and 5 out of 394 pups, respectively, had cleft palates, while there were none in any of the other groups. In 143 historical control groups with 2975 litters (dated 1971 to 1984), 7 out of 32 354 pups showed this specific malformation.

The NOAEL for maternal toxicity in these two tests was 10 mg/kg bw per day on the basis of reductions in body-weight gain at 30 mg/kg bw per day. The NOAEL for offspring toxicity was 50 mg/kg bw per day on the basis of cleft palates at 75 mg/kg bw per day (Machemer, 1976b).

Groups of 20–22 mated female FB30 (Long Evans) rats were exposed to triadimefon (purity not reported) at a concentration of 0, 0.014, 0.033 or 0.114 mg/l for 6 h per day on 10 consecutive days (day 6 to day 15 of gestation) by inhalation. On day 20 of gestation, the fetuses were removed by caesarian section. This is a pre-GLP study and no statement of QA was provided. At concentrations of ≥0.033 mg/l, the body-weight gain of the dams was reduced. There was no other evidence of effects on maternal, embryonic, or developmental toxicity. The NOAEC was 0.014 mg/l on the basis of body-weight effects in dams at 0.033 mg/l (Machemer & Kimmerle, 1976).

Rabbits

Groups of 10–13 mated female Himalayan rabbits were given triadimefon (purity not reported) at a dose of 0, 5, 15 or 50 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 29 of gestation, the fetuses were removed by caesarian section. This is a pre-GLP study and no statement of QA was provided. There was no evidence of maternal

or developmental toxicity. The NOAEL was 50 mg/kg bw per day, the highest dose tested (Machemer, 1976a).

Groups of 12 mated female Himalayan rabbits were given triadimefon (purity, 93.5%) at a dose of 0, 10, 30 or 100 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 29 of gestation, the fetuses were removed by caesarian section. There was no report on compliance of this study with any GLP standards and no statement of QA was provided. At 100 mg/kg bw per day, dams had changed faeces consistency, diarrhoea, and swollen and inflammated external vaginas. At doses of ≥30 mg/kg bw per day, the animals showed severely reduced body-weight gain, resulting in body-weight loss at the highest dose (Table 18).

At the highest dose, three animals showed complete resorption of their litters, with one in the control group and none in any other dosed group. One of 53 pups at the highest dose showed multiple malformations, although a relation to treatment is unlikely.

The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of body-weight changes of dams at 30 mg/kg bw per day. The NOAEL for offspring toxicity was 30 mg/kg bw per day on the basis of increased resorptions at 100 mg/kg bw per day (Roetz, 1982).

Groups of 20 American Dutch rabbits were given triadimefon (purity, 94.3%) at a dose of 0, 20, 50 or 120 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 28 of gestation, the fetuses were removed by caesarian section. This study complied with OECD guidelines for GLP.

At the highest dose, reduced feed consumption and a loss in body weight was found (Table 19). Fetuses in the group receiving the highest dose showed delayed ossification in

Table 18. Mean maternal body-weight gain in a study of developmental toxicity in Himalayan rabbits given triademefon by gavage

Dose (mg/kg bw per day)	Maternal body-weight gain (g)
0	39.1
10	47.5
30	4.6
100	−64.3

From Roetz (1982)

Table 19. Mean maternal body-weight gain in a study of developmental toxicity in American Dutch rabbits given triademefon by gavage

Dose (mg/kg bw per day)	Mean maternal body-weight gain (g)		
	Days 6–10	Days 6–18	Days 0–28
0	20	120	220
20	10	70	210
50	0	110	280
120	−80	50	270

From Clemens & Hartnagel (1990)

Table 20. Mean maternal body-weight gain in a study of developmental toxicity in American Dutch rabbits given triademefon by gavage

Dose (mg/kg bw per day)	Mean maternal body-weight gain (g)	
	Days 6–19	Days 0–28
0	170	310
40	70	260
60	80	290
80	70	290

From Clemens et al. (1991)

skeletal elements, malformations of caudal vertebrae and of spinous elements of the scapula (59 out of 118 fetuses, 50%) and cleft palates (2 out of 118 fetuses, 1.7%). The incidence of irregular spinous process of the scapula was also significantly increased (32 out of 121 fetuses, 26.4%) in the group receiving triademefon at a dose of 50 mg/kg bw per day. In 21 historical control groups with 335 litters (dated 1982 to 1988), only 2 out of 2034 pups had cleft palates and 18 out of 602 pups had irregularly formed scapulae. The NOAEL for offspring toxicity in this study was 20 mg/kg bw per day on the basis of scapula malformations at 50 mg/kg bw per day (Clemens & Hartnagel, 1990).

Groups of 20 American Dutch rabbits were given triadimefon (purity, 92.9%) at a dose of 0, 40, 60 or 80 mg/kg bw per day by gavage from day 6 to day 18 of gestation. On day 28 of gestation, the fetuses were removed by caesarian section. This study complied with OECD requirements for GLP.

All dosed animals had decreased body-weight gain (Table 20) and a slight increase in the incidence of reticulo-endothelial hyperplasia in the spleen was found. Scapula malformations were found in fetuses of all dosed groups (13.4%, 19.3% and 26.7% in the groups receiving the lowest, intermediate and highest doses respectively; for data on historical control groups, see above) and delayed ossification in skeletal elements at doses of ≥60 mg/kg bw per day. The uncommon finding of umbilical hernia was observed in one out of 119 fetuses at 60 mg/kg bw per day and in two out of 105 fetuses at 80 mg/kg bw per day. In this study, no NOAEL for maternal and developmental toxicity could be established (Clemens et al., 1991).

5.6 Special studies

(a) Neurotoxicity

Groups of 12 male and 12 female Wistar rats were given a single dose of triadimefon (purity, 95.8%) at 0, 2, 35 or 600 (males) or 400 (females) mg/kg bw by gavage and then observed for 14 days. This study complied with OECD requirements for GLP.

One male and four females in the group receiving the highest dose died within 2 days after dosing and the body-weight gain in this group was decreased. Male animals at the intermediate and the highest dose and females at the highest dose showed stereotypic behaviour, self mutilation and other signs of general toxicity. In the functional observational battery (FOB), animals at the intermediate or the highest dose showed effects shortly after dosing (most pronounced at 40 min), including affected posture and gait, increased activity, searching and cleaning gestures and increased rearing incidence. Overall, the effects on

FOB were reversible within 14 days. Although attenuated, the increased open-field rearing remained in males. The NOAEL was 2 mg/kg bw per day on the basis of signs of neurotoxicity at 35 mg/kg bw per day (Dreist & Popp, 1996a).

Groups of 18 male and 18 female Wistar rats were fed diets containing triadimefon (purity, 95.8%) at a concentration of 0, 50, 800 or 2200 ppm for 13 weeks. At the end of this period, six animals of each sex per group were terminated for neuropathology examination and the remaining animals were fed basal diet and observed for reversibility of any effects for another 4 weeks (males) or 10 weeks (females). This study complied with OECD requirements for GLP. The average intake of triadimefon was 0, 3.4, 54.6 and 150 mg/kg bw per day in males and 0, 4.3, 68.7 and 190 mg/kg bw per day in females. Intake was calculated according to food intake of the control animals, since a prominent increase in food consumption was observed in males at the highest dose (28% increased cumulative food intake, on the basis of grams/kg bw) and intermediate dose, and females at the highest dose (cumulative food intake was increased by 26% and 102%, respectively) which probably resulted from increased activity of the animals.

Body-weight gain was reduced in males at the intermediate (−5%) and highest doses (−13%) and in females at the highest dose (−15%). Males in the group receiving the highest dose and females in the groups receiving the intermediate and highest doses showed increased motility lasting for several weeks during the recovery period. Hyperactivity, indicated by effects on posture, increased rearing in the open field and pacing, was observed in animals of both sexes at the intermediate and highest doses. Most of the effects were reversible or attenuated in the recovery period.

The NOAEL was 50 ppm, equivalent to 3.4 mg/kg bw per day, on the basis of signs of neurotoxicity at 800 ppm (Dreist & Popp, 1996b).

There are several publications reporting on neurotoxicity induced by triadimefon in rats, as indicated by increased locomotor activity and stereotypical behaviour changes. These include studies in which triadimefon was administered orally, as well those in which it was administered by intraperitoneal application, from a dose of tens to a few hundred milligrams per kg bw per day. The magnitude of the effects and the time to recovery were related to dose (Crofton et al., 1988; Moser & MacPhail, 1989; Walker et al., 1990).

In a comparative study of neurotoxicity to reveal structure–activity relationships, male Long Evans rats were treated with one of 14 triazole fungicides or structurally related compounds. Eight to twelve animals per group received triadimefon or triadimenol at a dose of 0, 50, 100, 200 or 400 mg/kg bw. Signs of neurotoxicity were restricted to triadimefon and triadimenol and were reported as hyperactivity, which was statistically significant at the intermediate and the highest doses (Crofton, 1996).

Several studies suggest that the mechanism by which triadimefon causes neurotoxicity is via its potentiation of dopaminergic activity. In vitro, it was shown that triadimefon and triadimenol have a significant dopamine transporter-binding capacity, no dopamine-releasing function in the striatum, but a dopamine uptake-inhibiting effect in striatal synaptosomal preparations (Walker & Mailman, 1996; Ikaiddi et al., 1997).

In another study in rats, it was shown that the animals developed tolerance to triadimefon-induced enhanced locomotor and stereotypy behavioural patterns, since a new

challenge posed 14 days after the first of 12 consecutive exposures did not generate a response. This was also true in a similarly designed test for cross-sensitization with cocaine. These findings were accompanied by significant changes in dopaminergic biochemistry, which were interpreted by the study authors as adaptive responses to both single and repetitive exposures to triadimefon (Hill et al., 2000).

In a subsequent study on neurotoxicity in mice treated with triadimefon, animals were pretreated with α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA), *N*-methyl-D-aspartate (NMDA) and dopamine D1 and D2 receptor antagonists before they were exposed to triadimefon. Pretreatment with AMPA, NMDA or dopamine D1 receptor antagonists prevented the animals from developing behavioural changes, but the dopamine D2 receptor antagonist was inactive in this respect. The authors concluded that in addition to effects on dopamine re-uptake, AMPA, NMDA and dopamine D1 receptors are also involved in the development of triadimefon-mediated neurotoxicity (Reeves et al., 2004).

Male Bor:CFW1 mice and male Bor:WISW rats were tested for several pharmacological reactions after single oral exposures to triadimefon (purity, 92.6%) at a dose of 0, 0.3, 1.0 or 3.0 mg/kg bw.

Mice were examined for effects on hexobarbital anaesthesia, central coordination capability, analgesia, convulsion, anti-convulsion, traction capability, catalepsy, locomotor inhibition and spontaneous motility, and rats were tested for catalepsy, lingomandibular reflex and neuromuscular transmission. The only finding was a slight increase in spontaneous motility in mice of all dosed groups, which was not related to dose (Polacek, 1983b).

Although there is evidence from studies of acute toxicity and from longer-term studies specifically designed to identify such end-points that triadimefon has effects on the central nervous system, the majority of studies with repeated doses did not report such effects. Because of the age of the studies and the lack of information about monitoring of the animals, it is not clear whether such signs may have been present but were not noted or reported.

(b) Metabolites of triadimenol and triadimefon in rats

Owing to the close structural relationship between triadimenol and triadimefon, KWG 1342, KWG 1640 and KWG 1323 were identified as metabolites of triadimenol as well as of triadimefon in rats. To a very minor extent, free triazole is mentioned as a minor metabolite of triadimenol in rats. There was no such mention for triadimefon. The toxicity of triazole is discussed in section 7 (metabolites in plants).

In studies of acute oral toxicity, the LD_{50} for hydroxytriadimenol (KWG 1342) was >1000 mg/kg bw in fed female rats and >5000 mg/kg bw in fasted male rats (Heimann, 1985b), while the LD_{50} for carboxytriadimenol (KWG 1640) was >1000 mg/kg bw in male and female fasted rats (Heimann, 1985c) and hydroxytriadimefon (KWG 1323) had an LD_{50} of >5000 mg/kg bw in male and female fasted rats (Heimann, 1985a).

6. Observations in humans

Persons working with pure triadimefon showed no effects attributable to possible exposure (Kehrig & Steffens, 2003).

7. Metabolites of triazole fungicides in plants

In this section, the toxicity of the plant metabolites triazole, triazolyl alanine and triazole acetic acid is evaluated. Triazole was also identified in rats as a minor metabolite of triadimenol. Triazolyl alanine and triazole acetic acid are also produced from propiconazole by plants, but not by mammals (see propiconazole, p •• this volume).

A complete degradation of the chemical structure of triadimenol and triadimefon and formation of 4-chlorophenol and 1,2,4-triazole can occur in the soil. If 1,2,4-triazole is then taken up by the plant, it is conjugated by an enzymatic reaction with serine to form triazole alanine, which can be further transformed into triazole hydroxyl propanoic acid and triazole acetic acid. This chain of reactions can also occur in the soil.

7.1 Triazole

(a) Biochemical aspects

(i) Absorption, distribution, excretion and metabolism

Groups of two male and two female Sprague-Dawley rats were given ^{14}C-labelled 1,2,4-triazole as a single dose at 0.08, 9.8 or 173 mg/animal by gavage. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

Irrespective of the dose, about 90% of the administered dose was excreted in the urine and 10% in the faeces. Up to 98% of the dose was excreted in the first 48 h and at the final termination after 7 days the limits of quantification (0.002, 0.2 and 4 ppm, respectively, for the three doses, in increasing order) in tissues were exceeded only in fat (4.46 ppm in one female), testes (4.79 mg/kg in one male) and erythrocytes (8.57 ppm in one male) (Lai & Simoneaux, 1986c).

Groups of five male Sprague-Dawley rats were given ^{14}C-labelled 1,2,4-triazole as single doses at 0.1 and 100 mg/kg bw administered intravenously, 1 mg/kg bw administered orally, or 1 mg/kg bw administered intraduodenally. This is a pre-GLP study and no statement of QA was provided.

After oral administration, absorption of the substance was nearly 100%. After intravenous dosing, 50%, 1.5% and 0.3% was recovered in the body after 8 h, 3 days and 6 days, respectively. After oral or intravenous administration, only 0.1% of the administered dose was found in the exhaled air. Irrespective of the route of administration, 92–94% of the dose was excreted in the urine and 3–5% in the faeces. Studies with bile-duct fistulated rats showed 12% excretion in the bile, suggesting that the substance is reabsorbed. On day 6 after dosing, tissue concentrations were all near the limit of detection (Weber et al., 1978).

Ten male Sprague-Dawley rats were given ^{14}C-labelled 1,2,4-triazole as a single oral dose at 10 mg/kg bw per day and urine was analysed for metabolites. This is a pre-GLP study and no statement of QA was provided.

Of the excreted radiolabel, 90% was unchanged ^{14}C-labelled 1,2,4-triazole; other metabolites were not identified (Ecker, 1980).

Table 21. Acute toxicity of triazole

Species	Strain	Sex	Route	Purity (%)	LD_{50} (mg/kg bw)	LC_{50} (mg/l of air)	Reference
Rat	Crl:CD	Male	Oral	92.8	500–5000	—	Procopio & Hamilton (1981)
Rat	Wistar	Males and females	Oral	NR	1649	—	Thyssen & Kimmerle (1976a)
Rat	Wistar	Male	Dermal	NR	4200	—	Thyssen & Kimmerle (1976a)
Rat	Wistar	Female	Dermal	NR	3192	—	Thyssen & Kimmerle (1976a)
Rat[a]	Wistar	Male	Inhalation	NR	—	NA	Thyssen & Kimmerle (1976a)
Mouse[a]	NMRI	Male	Inhalation	NR	—	NA	Thyssen & Kimmerle (1976a)
Rabbit	NZW	Male	Dermal	92.8	200–2000	—	Procopio & Hamilton (1981)

NA, not applicable; NR, not reported; NZW, New Zealand White
[a]No effects observed; mice were exposed for 6 h and rats for 4 h; concentrations of triazole in air not given

(b) Toxicological studies

(i) Acute toxicity

The acute toxicity of triazole is summarized in Table 21.

(ii) Dermal and ocular irritation

In a study in New Zealand White rabbits, the triazole proved to be slightly irritating to the skin and the eyes (Procopio & Hamilton, 1981). In another study in New Zealand White rabbits, no effect on skin but strong irritation of the eyes was reported (Thyssen & Kimmerle, 1976a). Additionally, no effect on skin of humans was observed in this study.

(iii) Dermal sensitization

Triazole was not sensitizing to the skin of guinea-pigs in the Magnusson-Kligman maximization test. This study complied with the OECD requirements for GLP. Ten animals of each sex were dosed intradermally with 0.1 ml of a 10% formulation of triazole and then 1 week later topically with a 75% formulation of triazole. The challenge with a 75% formulation was performed 2 weeks after the dermal application (Frosch, 1998).

(iv) Short-term studies of toxicity

Rats

Groups of 15 male and 15 female Wistar rats were fed diets containing triazole (purity, 99.6%) at a concentration of 0, 100, 500 and 2500 ppm for 3 months. The average daily intakes of triazole were 0, 7.8, 37.9 and 212 mg/kg bw per day for males and 0, 10.2, 54.2 and 267 mg/kg bw per day for females. This is a pre-GLP study and no statement of QA was provided.

At 2500 ppm, body-weight gain was reduced in both sexes as was (temporarily) the food intake. In males at the highest dose, increased accumulation of fat in the liver and significantly lower haemoglobin concentration, erythrocyte volume fraction, mean corpuscular volume and mean corpuscular haemoglobin were observed.

The NOAEL was 500 ppm, equal to 37.9 mg/kg bw per day, on the basis of effects on body < weight, liver and blood at 2500 ppm (Bomhard et al., 1979).

Table 22. Studies of genotoxicity with triazole

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation[a]	*S. typhimurium* TA1535, TA1537, TA100, TA98	10–5000 µg/plate ±S9, in DMSO	99.7	Negative	Poth (1989)
Reverse mutation[b]	*S. typhimurium* TA1535, TA1537, TA100, TA98	100–7500 µg/plate ±S9, in DMSO	92.8	Negative	Melly & Lohse (1982)

DMSO, dimethylsulfoxide
[a] Cytotoxicity was observed at ≥1000 µg/plate
[b] Cytotoxicity was observed at ≥2000 µg/plate

(v) Genotoxicity

The results of studies of genotoxicity with triazole are summarized in Table 22.

(vi) Reproductive toxicity: developmental toxicity

Rats

In a comparative study of teratology in vitro, rat embryos were exposed to the antifungals flusilazole and fluconazole and to 1,2,4-triazole. This study did not comply with GLP. Unlike flusilazole and fluconazole, 1,2,4-triazole did not induce malformations in the branchial apparatus (Menegola et al., 2001).

In a comparative study of reproductive toxicity, Wistar rats were exposed to a set of substances with known effects on reproduction and development. 1,2,4-Triazole served as one of the negative controls in this study. This study did not comply with GLP requirements. As was expected, 1,2,4-triazole showed no effects on either reproduction or on development (Wickramaratne, 1987).

Groups of 25 mated female Wistar (Bor:WISW) rats were given 1,2,4-triazole (purity, 94%) at a dose of 0, 100 or 200 mg/kg bw per day by gavage from day 6 to day 15 of gestation. On day 20 of gestation, the fetuses were removed by cesarean section. This study complied with OECD requirements for GLP. This study supplemented a previous study of the same design that did not show any effects on fetuses at doses of 10, 30 and 100 mg/kg bw per day (Renhof, 1988b).

At the highest dose, the body-weight gain of dams was reduced. In the fetuses at ≥100 mg/kg bw per day, reduced body weights and higher incidences of undescended testicles (controls, 0.8%; 100 mg/kg bw, 4.9%; and 200 mg/kg bw, 4.3%) were reported, while at 200 mg/kg bw per day, increased implantation losses, reduced viability of the fetuses and increased malformations of the hind legs (2.9% versus 0% in the two other groups) and cleft palates (2.9% versus 0% in the two other groups) were observed. In historical controls of this strain, only two out of 13 892 fetuses (0.01%) had cleft palates and 75 (0.54%) had limb malformations (limb and type of malformation not further specified).

A NOAEL could not be identified in this study. Therefore, the NOAEL for developmental effects can be considered to be 30 mg/kg bw per day, from the first study (Renhof, 1988a).

In vitro

In a study on the kinetics of aromatase enzymes in granulosa cells in vitro, substituted triazole derivatives were found to be potent inhibitors of aromatase. 1,2,4-Triazole was found to give essentially negative results in this assay for inhibitors (Wickings et al., 1987).

7.2 Triazolyl alanine

The toxicity of triazolyl alanine was evaluated by the Meeting in 1989 (Annex 1, reference 58).

(a) Biochemical aspects

(i) Absorption, distribution, excretion and metabolism

In a balance study, groups of four male and four female Tif:RAIf rats were given ^{14}C-labelled triazolyl alanine (purity, >99%) at a dose of 0.5 or 50 mg/kg bw by gavage. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

At both doses, 95–105% of the administered dose was excreted within 24 h. Seven days after administration, 2–12% of the administered dose was excreted in the faeces, 88–108% in the urine and <1% in the exhaled air. At the lower dose, no radiolabel was detectable in tissues after 168 h, while at the higher dose radiolabel was detected at a concentration of <0.02 ppm. Electrophoretic characterization revealed that 86% of the administered dose was excreted unchanged (Hamboeck, 1983a).

In another study of balance and metabolism, groups of two male and two female Sprague-Dawley rats were given ^{14}C-labelled triazolyl alanine (purity, >99%) at a dose of 0.56, 54.4 or 994 mg/kg bw by gavage. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

At all doses, the renal excretion was 83%, while at increasing doses 16.1%, 6.2% and 7.7%, respectively, of the radiolabel was excreted in the faeces. Within the first 48 h, 97.4%, 87.3% and 88.2%; respectively, of the radiolabel was excreted at increasing doses. At final termination, with a few exceptions the tissue residue levels were below the limit of quantification at all doses.

In thin-layer chromatography performed on samples of urine, only two radioactive zones were identified. On comigration analyses it was estimated that 82–93% of the administered dose was excreted as unchanged triazolyl alanine and 13–30% as *N*-acetyltriazolyl alanine (Lai & Simoneaux, 1986b; Lai & Simoneaux, 1986e).

The findings of the above study (Lai & Simoneaux, 1986b; Lai & Simoneaux, 1986e) were generally confirmed in a similar study using nuclear magnetic resonance (NMR) and mass spectrometry (MS) for analyses. Additionally, both triazolyl alanine and *N*-acetyltriazolyl alanine were found at a level of approximately 1% in the faeces of rats (Hamboeck, 1983b).

Table 23. Acute toxicity of triazolyl alanine

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Purity (%)	References
Mouse	NMRI	Males and females	Oral, fasted	>5000	"Pure"	Mihail (1986)
Rat	Wistar	Males and females	Oral, fasted and fed	>5000	"Pure"	Mihail (1986)
Rat	Not reported	Males and females	Oral	>2000	Not reported	Henderson & Parkinson (1981)
Rat	Wistar	Males and females	Intraperitoneal	>5000	"Pure"	Mihail (1986)

(b) Toxicological studies

(i) Acute toxicity

The acute toxicity of triazolyl alanine is summarized in Table 23.

(ii) Short-term studies of toxicity

Rats

Groups of 10 male Wistar rats were given drinking-water containing triazolyl alanine (purity, approximately 100%) at a concentration of 0, 3000 or 10 000 mg/l for 2 weeks. The average daily intakes of triazolyl alanine were 0, 448 and 1490 mg/kg bw per day. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

There were no treatment-related findings related to appearance, behaviour, body-weight gain or gross examination of organs.

The NOAEL was 10 000 mg/l, equal to 1491 mg/kg bw per day, the highest dietary concentration tested (Bomhard, 1982).

Groups of 20 male and 20 female Wistar rats were given triazolyl alanine (purity, described as "pure") at a dose of 0, 25, 100 and 400 mg/kg bw per day by gavage for 4 weeks followed by a 4-week recovery period. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

There were no treatment-related findings after haematological, clinical-chemical, gravimetric, macroscopic and histopathological examinations.

The NOAEL was 400 mg/kg bw per day, the highest dose tested (Mihail & Vogel, 1983).

Groups of 20 male and 20 female Wistar (Bor:WISW) rats were fed diets containing triazolyl alanine (purity, 97.5%) at a concentration of 0, 1250, 5000 or 20 000 ppm for 3 months. The average daily intakes of triazolyl alanine were 0, 90, 370 and 1510 mg/kg bw per day for males, and 0, 100, 400 and 1680 mg/kg bw per day for females. This study did not comply with GLP requirements, but was supervised by the internal QA unit.

At the highest dose, the body-weight gain in males was slightly reduced relative to that in controls. There were no other treatment-related findings.

The NOAEL was 5000 ppm, equal to 370 mg/kg bw per day, on the basis of impairment of body-weight gain in males at 20 000 ppm (Maruhn & Bomhard, 1984).

Dogs

Groups of four male and four female beagle dogs were fed diets containing triazolyl alanine (purity, 97.5%) at a concentration of 0, 3200, 8000 or 20 000 ppm for 13 weeks. The average daily intakes of triazolyl alanine were 0, 119, 291 and 690 mg/kg bw per day (calculated from the daily intakes per animal of 0, 1185, 2914 and 6900 mg and a default body weight of 10 kg). This study did not comply with GLP requirements, but was supervised by the internal QA unit.

At the highest dose, the body-weight gain and food consumption of females was reduced. There were no other treatment-related findings.

The NOAEL was 3200 ppm, equivalent to 139 mg/kg bw per day, on the basis of impairment of body-weight gain at 8000 ppm (Keutz & Groening, 1984).

(iii) Genotoxicity

The results of studies of genotoxicity with triazolyl alanine are summarized in Table 24.

Table 24. Studies of genotoxicity with triazolyl alanine

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation[a]	*S. typhimurium* TA1535, TA1537, TA100, TA98, TA102	20–5000 µg/plate ±S9, in DMSO	97.4	Negative	Deparade (1986)
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA1538, TA100, TA98	20–12 500 µg/plate ±S9, in DMSO	NR	Negative	Herbold (1983b)
Reverse mutation	*E. coli* WP2 *uvra* and *S. typhimurium* TA1535, TA1537, TA100, TA98	312.5–5000 µg/plate ±S9, in DMSO	>96	Negative	Hertner (1993)
CHO/HGPRT mutation assay	Chinese hamster ovary cells	500–10 000 µg/ml ±S9	97.4	Negative	Dollenmeier (1986)
DNA damage	*E. coli* polA1+ and polA1−	62.5–1000 µg/plate ±S9, in DMSO	NR	Negative	Herbold (1983a)
Rec assay	*Bacillus subtilis* H17 (rec^+) and M45 (rec^-)	20–1000 µg/disk ±S9	>96	Negative	Watanabe (1993)
Unscheduled DNA synthesis	Primary rat hepatocytes from a male rat	0.25–10 000 µg/ml	97.5	Negative	Puri (1986)
Transformation	BALB/3T3	62.5–1000 µg/ml ±S9	97.4	Negative	Beilstein (1984)
Transformation	BHK 21C13	500–8000 µg/ml −S9 1000–16 000 µg/ml +S9	"No impurities identified"	Positive Positive	Richold et al. (1981)
In vivo					
Micronucleus formation	Bone marrow erythroblasts of male and female Chinese hamsters	5000 mg/kg bw, orally	97.4	Negative	Strasser (1986)
Micronucleus formation	Bone marrow erythroblasts of male CBC F1 mice	2500 and 5000 mg/kg bw, intraperitoneally	NR	Negative	Watkins (1982)
Micronucleus formation	Bone-marrow erythroblasts of male and female Bor:NMRI mice	8000 mg/kg bw, orally	NR	Negative	Herbold (1982)

DMSO, dimethyl sulfoxide; NR, not reported; S9, 9000 × *g* supernatant of rodent liver

[a] Precipitations at concentrations >78 µg/plate

(iv) Reproductive toxicity

Multigeneration studies

In a two-generation study in AP rats, groups of 6 males and 12 females were fed diets containing triazolyl alanine (purity, 48%; at the beginning of the study a purity of >90% was assumed) at a concentration of 0, 150, 625, 2500 or 10 000 ppm. There was no report on compliance of this study with any GLP standards and no statement of QA was provided. The pretreatment period before the first mating was 42 days.

In the group receiving the highest dose, the mean litter body weight on postnatal day 1 was slightly reduced, but returned to normal on postnatal day 5. In the parent females in the group receiving the highest dose, a tendency to prolonged intervals in the estrus cycle was observed.

The NOAEL was 2500 ppm on the basis of pup birth weight effects and possible effects on the estrus cycle at 10 000 ppm (Birtley, 1983).

In a two-generation study in Alpk:AP rats, groups of 15 males and 30 females were fed diets containing triazolyl alanine (purity, 97.8%) at a concentration of 0, 500, 2000 or 10 000 ppm. The pretreatment period before the first mating was 84 days. This study complied with the FIFRA requirements for GLP.

There was a slight reduction in birth weights of pups in the F_{1b} and F_{2a} generations at 10 000 ppm.

The NOAEL was 2000 ppm (Milburn et al., 1986).

Developmental toxicity

Groups of 24 mated female Alpk:AP rats were given triazolyl alanine (purity, 94.8%) at a dose of 0, 100, 300 or 1000 mg/kg bw per day by gavage from day 7 to day 16 of gestation. On day 22 of gestation, the fetuses were removed by cesarean section. This study did not comply with GLP requirements but was supervised by the internal QA unit.

A slight increase in non-ossification of odontoid processes was observed at 300 mg/kg bw per day, while an increase in retarded ossification of different bones was observed at 1000 mg/kg bw per day.

The NOAEL was 100 mg/kg bw per day on the basis of slight effects on skeletal development at 300 mg/kg bw per day (Clapp et al., 1983).

7.3 Triazole acetic acid

(a) Biochemical aspects

(i) Absorption, distribution, excretion and metabolism

In a balance study, groups of two male and two female Sprague-Dawley rats were given ^{14}C-labelled triazole acetic acid (purity, >99%) at a dose of 0.58, 58.6 or 1030 mg/kg bw by gavage. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

Irrespective of sex and dose, 90.6–102.5% of the administered substance was excreted in the urine and 3.1–4.3% in the faeces. Within the first 48 h, excretion was nearly complete and at 7 days after dosing, residues in tissues (plasma and testes) were found to be only incidental above the level of quantification (Lai & Simoneaux, 1986a).

In a study of metabolism, groups of two male and two female Sprague-Dawley rats were given ^{14}C-labelled triazole acetic acid (purity, >99%) at a dose of 0.58, 58.6 or 1030 mg/kg bw by gavage. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

In the urine, unchanged triazole acetic acid was the only radioactive substance found (Lai & Simoneaux, 1986d).

(b) Toxicological studies

(i) Acute toxicity

The acute toxicity of triazole acetic acid is summarized in Table 25.

(ii) Short-term studies of toxicity

Rats

Groups of five male and five female RAIf rats were fed diets containing triazole acetic acid at a concentration of 0, 100, 1000 or 8000 ppm for 14 days. The average daily intakes of triazole acetic acid were 0, 11, 103 and 788 mg/kg bw per day for males and 0, 10, 97 and 704 mg/kg bw per day for females. There was no report on compliance of this study with any GLP standards and no statement of QA was provided.

There were no treatment related findings.

The NOAEL was 704 mg/kg bw per day, the highest dose tested (Thevenaz, 1986).

(iii) Genotoxicity

The results of studies of genotoxicity with triazole acetic acid are summarized in Table 26.

Table 25. Acute toxicity of triazole acetic acid

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	Purity (%)	Reference
Rat	Tif:RAIf	Males and females	Oral	>5000	>99	Thevenaz (1994)

Table 26. Studies of genotoxicity with triazole acetic acid

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation	*S. typhimurium* TA1535, TA1537, TA100, TA98	20–5120 µg/plate ±S9	>99	Negative	Deparade (1984)

Comments

Triadimenol

In rats, radiolabelled triadimenol is rapidly absorbed from the gastrointestinal tract, with radioactivity reaching peak concentrations in most tissues between 1 h and 4 h after dosing. Up to 90% of the administered dose was excreted, with an elimination half-life for the radiolabel of between 6 h and 15 h. Excretion was essentially complete within 96 h. After 5–6 days, the amount of radioactivity in most organs was below the limits of quantification.

Renal excretion accounted for ≤21% of the orally administered dose in males and ≤48% in females. The remainder was found in the faeces. In bile-duct cannulated males 93% of the administered dose was recovered in the bile and only 6% in the urine. Thus a substantial amount of the administered dose undergoes enterohepatic recycling. Radioactivity in expired air was negligible.

Triadimenol was extensively metabolized, predominantly by oxidation of one of the *t*-butyl methyl groups to give hydroxy or carboxy derivatives. The putative intermediate triadimefon has not been isolated. Cleavage of the chloro-phenyl and the triazole group was of minor significance. In the urine and faeces most of the metabolites were not conjugated, but in bile the metabolites were found to be extensively glucuronidated.

Triadimenol has low to moderate acute toxicity. The acute oral LD_{50} both in mice and rats was in the range of 700 to 1500 mg/kg bw, with increasing toxicity for increasing isomer ratios A (1RS,2SR):B (1RS,2RS). This finding was supported by an oral LD_{50} of 579 mg/kg bw for isomer A and 5000 mg/kg bw for isomer B tested separately. In rats, the dermal LD_{50} was >5000 mg/kg bw and the LC_{50} upon inhalation was >0.954 mg/l of air (after an exposure of 4 h).

Triadimenol is not an ocular or dermal irritant in rabbits and is not a sensitizer in the maximization test in guinea-pigs.

In short-term studies in mice, rats and dogs, the main effect of triadimenol was on the liver.

In a study comparing the 80:20 and 60:40 isomer mixtures, rats were treated for 28 days by gavage. Both isomer compositions slightly increased motor activity at ≥45 mg/kg bw per day, and induced mixed function oxidase activity and reversibly increased liver weight at 100 mg/kg bw per day. In mice fed diets containing triadimenol at a concentration of 160 to 4500 ppm for 13 weeks, one out of ten males at 4500 ppm died. In both sexes at ≥1500 ppm, there were increased liver weights accompanied by increased alanine aminotransferase and AST activities. Reduced erythrocyte volume fraction and increased mean corpuscular haemoglobin concentration were observed in females at the highest dose. The NOAEL was 500 ppm, equal to 76.8 mg/kg bw per day.

In two 3-month feeding studies in rats, liver weights were increased at ≥600 ppm (<10% at 600 ppm), with cellular hypertrophy at 3000 ppm. Liver enzyme activities in serum were not increased. In one study, kidney and ovary weights were also increased at the highest dose of 2400 mg/kg. At the highest doses in both studies, there were slight changes in some haematology parameters. The lowest NOAEL after oral administration in the short-term

studies in rats was 600 ppm, equal to 39.6 mg/kg bw per day. In a 3-week study in rats treated by inhalation, no effects were observed at up to the highest dose of 2.2 mg/l of air.

In a 3-week study in rabbits, dermal application of triadimenol did not cause any dermal or systemic reactions at the highest dose tested, 250 mg/kg bw per day.

In a 3-month, a 6-month and a 2-year study, dogs were given diets containing triadimenol at concentrations of ≤2400 ppm. The only significant findings were decreased body-weight gain at 2400 ppm, liver and kidney weight increases at the highest doses and increased cytochrome P450 levels. The overall NOAEL was 600 ppm, equal to 21.1 mg/kg bw per day.

In two long-term studies, mice were given diets containing triadimenol at a concentration of ≤2000 ppm. In one study, Crl:CD-1(ICR)BR mice were kept for 80 weeks, and in the other study CF_1/WF 74 mice were kept for 2 years. At 2000 ppm, reduced body-weight gains were recorded and liver weights were increased, as were testes weights in one study. Additionally, liver enzyme activity was higher. In one study, histopathological examination of the liver showed more basophilic foci at ≥80 ppm, predominantly in males, but there was a poor dose–response relationship and similar values have been reported in control groups in other studies. Hepatocellular hypertrophy and single cell necrosis were found at ≥400 ppm. At 2000 ppm, additional histopathological changes to the liver were reported. At the intermediate dose, 400 ppm, but not at the highest dose, males had slightly more liver adenomas and carcinomas. There was no clear dose–response relationship, and values were within the historical control range of 6–17%. In females at the highest dose, two out of 50 animals had luteomas; this was within the range for historical controls of 0.9–10%. In the other study, females at the intermediate and highest dose had more liver adenomas and in both sexes at the highest dose, the incidences of liver hyperplastic nodules and thyroid cystic alterations were increased. The increase in liver adenomas is a common finding in mice, which is considered to be of questionable relevance for humans. The overall NOAEL was 500 ppm, equal to 140 mg/kg bw per day.

In a long-term feeding study in rats, reduced body-weight gain was found in both sexes at the highest concentration of 2000 mg/kg, as were changes in the weights of a number of organs, including spleen, lung and testes. However, there was a poor relationship with dose. In females, kidney, liver and ovarian weights were higher at the highest dose. In both sexes at 2000 ppm, the activities of liver enzymes (ALT and AST in both sexes and glutamate dehydrogenase in males) were slightly increased. At the highest dose, minor changes in haematology parameters were at the borderline of the physiological range at some time-points. There was no histopathological evidence for any non-neoplastic or neoplastic changes. The NOAEL was 500 ppm, equal to 25 mg/kg bw per day.

In a series of studies of genotoxicity in vitro and in vivo, triadimenol consistently gave negative results. The Meeting concluded that triadimenol is unlikely to be genotoxic.

In view of the lack of genotoxicity observed, and the finding of liver tumours only in female mice and only at concentrations at which liver toxicity was observed, the Meeting concluded that triadimenol is not likely to pose a carcinogenic risk to humans.

To study reproductive performance during exposure to triadimenol, two- and three-generation feeding studies were performed in rats given diets containing triadimenol at

concentrations of ≤500 ppm and ≤2000 ppm, respectively. In the study in which the higher doses were administered, matings in all three generations consistently showed reduced fertility at ≥500 ppm; in F_0 matings, this finding was observed at 125 ppm. Reduced viability was observed in F_1 pups of both matings at 2000 ppm, F_2 pups from the first mating at ≥500 ppm, and F_2 pups of the second mating at 2000 ppm. All F_3 pups from the first mating died at ≥500 ppm, but not those from the second mating. At 500 ppm, increased testicular and ovarian weights were observed in F_{1b} parents in the study in which lower doses were administered, and increased testicular weights in the F_{2b} parents at 2000 ppm. The lowest NOAEL in these studies was 100 ppm, equal to 8.6 mg/kg bw per day.

Several studies of developmental toxicity were performed in rats, over a dose range of 5 to 120 mg/kg bw per day. In one study, an increase in supernumerary lumbar ribs was found at ≥25 mg/kg bw per day, and in another study there was an increase in postimplantation losses at 120 mg/kg bw per day. In three out of the four studies, increased placental weights were noted at doses of 30 to 100 mg/kg bw per day. Such effects have been reported with other azoles. Triadimenol did not induce malformations in studies of developmental toxicity and clear NOAELs for developmental toxicity could be established; the lowest NOAEL was 15 mg/kg bw per day.

The NOAEL for offspring toxicity in rabbits was 4 mg/kg bw per day on the basis of slightly increased postimplantation losses at the maternally toxic dose of 200 mg/kg bw per day.

Clinical signs (general restlessness, alternating phases of increased and reduced motility, aggressivity) observed during tests for acute toxicity suggested possible effects on the central nervous system.

The Meeting concluded that the existing database on triadimenol was adequate to characterize the potential hazards to fetuses, infants and children.

A medical survey of personnel working in the production of triadimenol gave no indication of any substance-related effects.

Toxicological evaluation

Although a series of tests for acute neurotoxicity in mice were available, a NOAEL for triadimenol for neurotoxicity could not be identified because of technical shortcomings in these studies. As triadimenol is closely related to triadimefon in terms of chemical structure and toxicological effects, and in the view of the lack of sound studies of neurotoxicity with triadimenol, the Meeting concluded that studies of neurotoxicity performed with triadimefon could serve as a basis for derivating an ADI and an ARfD for triadimenol. This was supported by evidence for similar neurotoxic potential in a published study of acute toxicity with triadimenol and triadimefon.

The Meeting established an ADI of 0–0.03 ppm based on the NOAEL of 3.4 mg/kg bw per day for hyperactivity in a study of neurotoxicity with triadimefon in a 13-week feeding study in rats, and with a safety factor of 100.

The Meeting established an ARfD of 0.08 mg/kg bw on the basis of the NOAEL of 2 mg/kg bw for hyperactivity in a study of acute neurotoxicity in rats treated with triadimefon by gavage. A safety factor of 25 was applied because the effects were C_{max}-dependent and reversible (see comments on triadimefon).

Triadimefon

In a study on the absorption, distribution, metabolism and excretion of triadimefon in rats, the dose given and pretreatment with non-labelled triadimefon did not significantly affect excretion and metabolism patterns. In males about one third and in females about two thirds of the administered dose was excreted in the urine, and vice versa in the faeces. After 96 h, 2% of the radioactivity remained in females and 9% in males, with the highest residue concentrations found in liver and kidneys.

The metabolism of triadimefon starts either by direct oxidation of a *t*-butyl methyl group to the hydroxy or the carboxy compound with subsequent glucuronidation, or these steps are preceded by reduction of the keto group of triadimefon to the putative intermediate, triadimenol. Therefore, many of the metabolites found in triadimenol metabolism studies are also found with triadimefon. Nevertheless, the metabolism of triadimefon in rats provides a pathway for demethylation of the *t*-butyl group, which is not seen with triadimenol. This might be owing to very low biotransformation of triadimenol via triadimefon as intermediate.

The acute oral LD_{50} in mice and rats was in the range of 363 to 1855 mg/kg bw. The dermal LD_{50} was >5000 mg/kg bw and the LC_{50} on inhalation was >3.27 mg/l of air.

In rabbits, a few treatment-related effects including skin and eye irritation were recorded, but the irritation potential of triadimefon was very low. In guinea-pigs, technical-grade triadimefon of low purity was a sensitizer in the Büehler test for skin sensitization. However, purified triadimefon did not have any sensitizing potential in guinea-pigs in the Magnusson & Kligman maximization test, even after induction with technical-grade triadimefon of low purity.

In short-term studies in rats and dogs, the main effects of triadimefon were on the liver.

In three short-term studies in rats (treated by gavage at doses of ≤30 mg/kg bw per day for 30 days, by gavage at doses of ≤25 mg/kg bw per day for 4 weeks, and given diets containing triadimefon at concentrations of ≤2000 ppm for 12 weeks) the overall NOAEL was 150 mg/kg bw per day, the highest dose tested.

In two studies in dogs fed with diets containing triadimefon for 13 weeks and 2 years, the highest concentrations administered were 2400 ppm and 2000 ppm, respectively. Bodyweight decreases, relative liver weight increases and liver enzyme induction were observed predominantly in the group receiving the highest dose, and, in the short-term study only, there were also effects on haematology parameters. The overall NOAEL in these studies was 600 ppm, equal to 17.3 mg/kg bw per day, in the 2-year study.

The dermal application of triadimefon at 1000 mg/kg bw per day to rats for 3 weeks (6 h per day for 5 days per week) caused diffuse acanthosis at the application site and

increased activity and reactivity. The NOAEL was 300 mg/kg bw per day. The dermal application of triadimefon at 50 and 250 mg/kg bw per day to rabbits for 4 weeks (5 days per week) caused mild erythema at the application sites. Rats exposed by inhalation to triadimefon at 0.3 mg/l of air had reduced body-weight gain and increased liver weights.

In two 2-year feeding studies in mice, severely decreased body-weight gains, changes in several haematology parameters and increased liver weights and increased enzyme activity were observed at the highest dietary concentration of 1800 ppm. Starting at 300 ppm, histopathological changes, including nodular changes, hypertrophy and single cell necrosis, were found in the liver. These effects were more pronounced at the highest dose, and in one study an increase in hepatocellular adenomas was also reported. In the other study, a re-examination of histopathology slides led to re-classification of findings for adenomas and carcinomas. Owing to incomplete re-examination, a final conclusion on whether the incidences were increased or not was not possible. However, liver adenomas in the presence of liver toxicity in mice are generally not believed to be of toxicological concern for humans.

The lowest NOAEL was 50 ppm, equal to 13.5 mg/kg bw per day, on the basis of nodular changes and single cell necrosis in the liver at 300 ppm.

With the exception of behavioural changes and severe histopathological lesions in several organs observed in one study at the highest dose of 5000 ppm, the toxicological profile in two 2-year feeding studies in rats was very similar to that of the studies in mice. After 23 weeks of exposure to the highest dose at 5000 ppm, animals showed violent activity and refused the feed and became moribund. The surviving animals in this group were terminated at week 39. They showed haemorrhagic lesions in the stomach mucosa, blood-filled and dilated alveolar vessels, degenerative processes in proximal kidney tubules of females, atrophied spleens with signs of decreased haematopoesis, some giant spermatids in testes, and decreased haematopoesis in the bone marrow of males. At the lower dietary concentrations of 1800 and 500 ppm, reduced body-weight gains, increased liver weights and mildly increased liver enzyme activities were recorded. In one study, ovary weights were higher and adrenal weights lower. Mild effects on haematology were found in both studies. In one study at the highest dietary concentration of 1800 ppm, a marginal increase in thyroid cystic hyperplasias and more thyroid follicular adenomas (five versus zero for both sexes taken together) were found. When compared with historical controls, this effect was not significant. The overall NOAEL was 300 ppm, equal to 16.4 mg/kg bw per day.

In a series of studies of genotoxicity in vitro and in vivo, all results were consistently negative. The Meeting concluded that triadimefon is unlikely to be genotoxic.

In view of the lack of genotoxicity and the finding only of liver adenomas in mice and equivocal changes in thyroid follicular adenomas in rats at concentrations at which organ toxicity was observed, the Meeting concluded that triadimefon is not likely to pose a carcinogenic risk to humans.

In two related multigeneration studies, rats received diets containing triadimefon at concentrations of ≤1800 ppm. Maternal and pup weight development was reduced at doses of ≥300 ppm and, in the first generation at the highest dose, the viability of the pups was reduced. At the highest dose, two matings of the F_1 animals to give F_2 generation pups resulted in one female becoming pregnant in the first mating and none in the second. In the second study, again at 1800 ppm, the fertility of the F_0 generation was not affected, but that

of the F_1 generation was, albeit not to the same extent as in the first study. Viability and pup weights were reduced. In a cross mating in which only one sex was exposed to triadimefon, only the matings with exposed males gave significantly reduced fertility, correlating with reduced insemination indices. Therefore, reduced fertility seemed to have resulted mainly from impaired mounting willingness of exposed males. In males at the highest dose, the concentration of testosterone was double that in control males, and testes weights were increased. However, no correlation between individual testosterone concentrations and spermiograms and mating willingness was observed, although reduced mating willingness did appear to correlate with reduced body weight. It appears that prenatal, but not postnatal, exposure of males affected mating willingness. The lowest NOAEL was 50 ppm, equivalent to 3.75 mg/kg bw per day, based on a LOAEL of 1800 ppm for reproductive effects.

In studies of developmental toxicity in rats treated by inhalation (one study) and by gavage (two studies), inhalation exposure at air concentrations of ≤0.114 mg/l of air on day 6 to day 15 of gestation did not result in any findings indicative of developmental toxicity. In the studies of rats treated by gavage, however, supernumerary ribs in one study at 90 mg/kg bw per day, increased placental weights at 100 mg/kg bw per day, and cleft palates at doses of ≥75 mg/kg bw per day were found. These doses also reduced the body-weight gains of dams by ≤50% over the exposure period, but not when averaged over the whole gestation period. In four studies in rabbits, body-weight loss in dams was observed at a dose of ≥30 mg/kg bw per day. Over the dose range of 60 to 120 mg/kg bw per day, increased litter losses, and caudal vertebrae malformations and cleft palates were found either in one or the other study and delayed ossification and scapula malformations were observed in both studies. Additionally, in one study, the uncommon finding of umbilical hernia was recorded in pups at 60 and 80 mg/kg bw per day. Scapula deformations were also found at 40 mg/kg bw per day, the lowest dose tested in the study. Overall, the lowest NOAEL for offspring toxicity was 20 mg/kg bw on the basis of scapula deformations at 40 mg/kg bw in rabbits.

Several studies provide evidence that triadimefon has neurotoxic potential. In a study in which single doses of triadimefon were administered by gavage and in a 13-week feeding study, several signs of hyperactivity, increased motility and stereotypic behaviour were found. The NOAEL in the former study was 2 mg/kg bw on the basis of reversible neurotoxic effects at 35 mg/kg bw. These were considered to be C_{max}-dependent effects in view of the fact that a dose of 54.6 mg/kg bw per day in the short-term feeding study caused similar effects only after several days. The NOAEL for this study was 50 ppm, equivalent to 3.4 mg/kg bw. In a comparative study of acute neurotoxicity in Long Evans rats treated by gavage with a group of 14 triazoles or structurally related compounds, hyperactivity at 100 mg/kg bw, but not at 50 mg/kg bw, was recorded for both triadimenol and triadimefon. In this study, the dose–response curves for triadimenol and triadimefon were very similar, suggesting a common mechanism of neurotoxicity.

The Meeting concluded that the existing database on triadimefon was adequate to characterize the potential hazards to fetuses, infants and children.

A medical survey of the personnel working in the production of triadimefon gave no indication of any substance-related effects.

Toxicological evaluation

The Meeting established an ADI of 0–0.03 mg/kg bw on the basis of the NOAEL of 3.4 mg/kg bw per day for hyperactivity in a study of neurotoxicity in rats fed with triadimefon and a safety factor of 100.

The Meeting established an ARfD of 0.08 mg/kg bw based on the NOAEL of 2 mg/kg bw for hyperactivity in a study of acute neurotoxicity in rats given triadimefon by gavage. A safety factor of 25 was used since the effects were C_{max}-dependent and reversible.

Plant metabolites of triadimefon, triadimenol and other triazole fungicides

Triazole, triazolyl alanine and triazole acetic acid are plant metabolites of several triazole fungicides, including triadimenol and triadimefon.

After oral administration of triazole, triazolyl alanine and triazole acetic acid to rats, these compounds are rapidly and completely absorbed. Urinary excretion is the main excretion pathway for ≥90% of the administered dose, and only a few percent are found in the faeces. Except for triazolyl alanine, which is metabolized to a minor extent to *N*-acetyltriazolyl alanine, these compounds are virtually not metabolized and are excreted unchanged. Owing to rapid and complete excretion, there is no potential for accumulation in the body for any of these plant metabolites.

The acute oral toxicity of all three compounds is low, with LD_{50}s of >5000 mg/kg bw, except for triazole, with an LD_{50} of 1649 mg/kg bw.

Only a few tests for genotoxicity have been performed on triazole and triazole acetic acid and all gave negative results. Triazolyl alanine was more extensively tested; only one test for cell transformation in vitro gave a positive result, while the results of another similar test and all other tests were negative.

In a 3-month feeding study in rats, triazole induced fat deposition in the liver and changes in haematological parameters at the highest dose of 2500 ppm. In 3-month feeding studies in rats and, the only effect of triazolyl alanine was to reduce body-weight gain at the highest dose of 20 000 ppm. No effects were recorded in a 2-week study in rats fed with triazole acetic acid at the highest dose of 8000 ppm.

In a study of developmental toxicity with triazole in rats, at ≥100 mg/kg bw per day fetuses showed increased incidence of undescended testicles and at 200 mg/kg bw per day malformations of the hind legs were found. In studies of reproductive and developmental toxicity with triazolyl alanine in rats, only very minor effects on pups, indicative of general toxicity, such as reduced birth weights and retarded ossification processes were found at high doses. There were no studies of reproductive and developmental toxicity with triazole acetic acid.

Since triazolyl alanine and triazole acetic acid were of low systemic toxicity and developmental effects with triazole occur at doses of ≥100 mg/kg bw per day, these metabolites were judged not to pose an additional risk to humans.

Triadimenol

Levels relevant to risk assessment of triadimenol#

Species	Study	Effect	NOAEL	LOAEL
Mouse	80-week study of toxicity and carcinogenicitya	Toxicity	500 ppm, equal to 140 mg/kg bw per day	2000 ppm, equal to 620 mg/kg bw per day
		Carcinogenicity	500 ppm, equal to 140 mg/kg bw per day	2000 ppm, equal to 620 mg/kg bw per day
Rat	2-year study of toxicity and carcinogenicity[a]	Toxicity	500 ppm, equal to 25 mg/kg bw per day	2000 ppm, equal to 105 mg/kg bw per day
		Carcinogenicity	2000 ppm, equal to 105 mg/kg bw per dayc	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	100 ppm, equal to 8.6 mg/kg bw per day	500 ppm, equal to 43.0 mg/kg bw per day
		Pup toxicity	100 ppm, equal to 8.6 mg/kg bw per day	500 ppm, equal to 43.0 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	25 mg/kg bw per day	60 mg/kg bw per day
		Embryo- and fetotoxicity	15 mg/kg bw per day	25 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	40 mg/kg bw per day	200 mg/kg bw per day
		Embryo- and fetotoxicity	40 mg/kg bw per day	200 mg/kg bw per day
Dog	13-week study of toxicity[a]	Toxicity	600 ppm equal to 21.1 mg/kg bw per day	2400 ppm equal to 85.9 mg/kg bw per day

See comments on triadimefon
[a] Diet
[b] Gavage
[c] Highest dose tested

Estimate of acceptable daily intake for humans

0–0.03 mg/kg bw

Estimate of acute reference dose

0.08 mg/kg bw

Studies that would provide information useful for the continued evaluation of the compound

Further observations in humans

Summary of critical end-points for triadimenol

Absorption, distribution, metabolism and excretion in animals	
Rate and extent of oral absorption	Rapid (peak within 1.5 h); >90%
Distribution	Widely distributed
Potential for accumulation	Low, half-lives of 6–15 h
Rate and extent of excretion	79–90% within 2 h
Metabolism	Very extensive; predominantly oxidation of *t*-butyl methyl group
Toxicologically significant compounds (animals, plants and the environment)	Triadimenol, triadimefon, triazole
Acute toxicity	
Rat, LD_{50}, oral	579–5000 mg/kg bw (varies with isomer composition)
Rat, LD_{50}, dermal	>5000 mg/kg bw
Rat, LC_{50}, inhalation	>0.95 mg/l
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Skin sensitization	Not sensitizing (Magnusson & Kligman maximization test)
Short-term studies of toxicity	
Critical effects	Liver toxicity (2-year study in dogs)
Lowest NOAEL	21.1 mg/kg bw
Genotoxicity	Negative results in vitro and in vivo
Long-term studies of toxicity and carcinogenicity	
Critical effects	Body and organ weight changes (2-year study in rats)
Lowest NOAEL	25 mg/kg bw
Carcinogenicity	Liver adenomas in female mice; unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Critical effects	Increased ovary and testes weights (rat)
Lowest reproductive NOAEL	8.6 mg/kg bw
Critical effects	Increased supernumerary lumbar ribs; not teratogenic (rat)
Lowest developmental NOAEL	15 mg/kg bw
Neurotoxicity/delayed neurotoxicity	
Critical effects at LOAEL	See triadimefon
Lowest NOAEL	See triadimefon
Other toxicological studies	Metabolites are of no greater toxicological concern than the parent
Medical data	No effects on health in manufacturing personnel

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.03 mg/kg bw	Rat, short-term study of neurotoxicity with triadimefon (see triadimefon)	100
ARfD	0.08 mg/kg bw	Rat, study of acute neurotoxicity with triadimefon (see triadimefon)	25

Triadimefon

Levels relevant to risk assessment of triadimefon

Species	Study	Effect	NOAEL	LOAEL
Mouse	21-month study of toxicity and carcinogenicity[a]	Toxicity	50 ppm, equal to 13.5 mg/kg bw per day	300 ppm, equal to 76 mg/kg bw per day
		Carcinogenicity	300 ppm, equal to 76 mg/kg bw per day	1800 ppm, equal to 550 mg/kg bw per day
Rat	105-week study of toxicity and carcinogenicity[a]	Toxicity	300 ppm, equal to 16.4 mg/kg bw per day	1800 ppm, equal to 114 mg/kg bw per day
		Carcinogenicity	1800 ppm, equal to 114 mg/kg bw per day[c]	—
	Two-generation study of reproductive toxicity[a]	Parental toxicity	300 ppm, equal to 22.8 mg/kg bw per day	1800 ppm, equal to 136.8 mg/kg bw per day
		Pup toxicity	300 ppm, equal to 22.8 mg/kg bw per day	1800 ppm, equal to 136.8 mg/kg bw per day
	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	30 mg/kg bw per day
		Embryo- and fetotoxicity	30 mg/kg bw per day	90 mg/kg bw per day
	Acute neurotoxicity[b]	Neurotoxicity	2 mg/kg bw	35 mg/kg bw
	13-week study of neurotoxicity[a]	Neurotoxicity	50 ppm, equivalent to 3.4 mg/kg bw per day	800 ppm, equivalent to 54.6 mg/kg bw per day
Rabbit	Developmental toxicity[b]	Maternal toxicity	10 mg/kg bw per day	30 mg/kg bw per day
		Embryo- and fetotoxicity	20 mg/kg bw per day	50 mg/kg bw per day
Dog	2-year study of toxicity[a]	Toxicity	300 ppm equal to 11.7 mg/kg bw per day	200 ppm equal to 48.8 mg/kg bw per day

[a] Diet
[b] Gavage
[c] Highest dose tested

Estimate of acceptable daily intake for humans

0–0.03 mg/kg bw

Estimate of acute reference dose

0.08 mg/kg bw

Studies that would provide information useful for the continued evaluation of the compound

Further observations in humans

Summary of critical end-points for triadimefon

Absorption, distribution, metabolism and excretion in animals	
Rate and extent of oral absorption	≥28% in females, ≥67% in males as urinary excretion
Distribution	Widely distributed in kidneys and liver
Potential for accumulation	Low
Rate and extent of excretion	90–98% excretion within 96 h
Metabolism	Very extensive; predominantly oxidation of t-butyl methyl group
Toxicologically significant compounds (plants, animals and the environment)	Triadimenol, triadimefon, triazole
Acute toxicity	
Rat, LD_{50}, oral	363–1855 mg/kg bw
Rat, LD_{50}, dermal	>5000 mg/kg bw
Rat, LC_{50}, inhalation	>3.27 mg/l
Rabbit, dermal irritation	Not irritating
Rabbit, ocular irritation	Not irritating
Skin sensitization	Technical-grade triadimefon is sensitizing, purified triadimefon is not sensitizing (Büehler, and Magnusson & Kligman maximization tests)
Short-term studies of toxicity	
Critical effects	Liver effects (dog)
Lowest NOAEL	17.3 mg/kg bw
Genotoxicity	Negative in vitro and in vivo
Long-term studies of toxicity and carcinogenicity	
Critical effects	Liver nodular changes, hypertrophy and single cell necrosis
Lowest NOAEL	13.5 mg/kg bw per day
Carcinogenicity	Liver adenomas in mice; unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Critical effects	Impaired reproductive performance (rat)
Lowest reproductive NOAEL	22.8 mg/kg bw per day
Critical effects	Scapula malformations at maternal toxic doses (rabbit)
Lowest developmental NOAEL	20 mg/kg bw per day
Neurotoxicity/delayed neurotoxicity	
Critical effects	Increased activity in study of acute neurotoxicity after gavage administration (rat)
Lowest NOAEL	2 mg/kg bw
Critical effects	Increased activity in short-term feeding study (rat)
Lowest NOAEL	3.4 mg/kg bw
Other toxicological studies	Metabolites are of no greater toxicological concern than the parent
Medical data	No effects on health in manufacturing personnel

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.03 mg/kg	Rat, short-term study of neurotoxicity	100
ARfD	0.08 mg/kg	Rat, study of acute neurotoxicity	25

References

Bayer CropScience AG (2003) Triadimenol dossier according to directive 91/414/EEC. Annex II, 5. Toxicology and Metabolism. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Becker, H., Frei, D., Vogel, W. & Terrier, C. (1987a) Embryotoxicity (including teratogenicity) study with KWG 0519 in the rat. Unpublished report No. R3988 from RCC, Research and Consulting Company AG, Itingen, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Becker, H., Mueller, E., Vogel, W. & Terrier, C. (1987b) Embryotoxicity (including teratogenicity) study with KWG 0519 in the rabbit. Unpublished report No. R4145 from RCC, Research and Consulting Company AG, Itingen, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Beilstein, P. (1984) CGA 131013 tech.—Transformation/liver-microsome test. Unpublished report No. 840324 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Birtley, R.D.N. (1983) Triazole alanine—preliminary reproduction study in the rat. Unpublished report No. CTL/L/470 from Imperial Chemical Industries PLC, Cheshire, UK. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E. (1982) THS 2212—preliminary subacute toxicity study on male rats, administration in the drinking water. Unpublished report No. 11253 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E. & Hahnemann, S. (1986) MEB 6447 (common name: triadimefon, the active ingredient of Bayleton). Carcinogenicity study on NMRI mice (21-month administration in the feed). Unpublished report No. 16719 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E. & Loeser, E. (1978) MEB 6447—chronic toxicity study on rats (two-year feeding experiment). Unpublished report No. 7707 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E. & Loeser, E. (1980) MEB 6447—chronic toxicity study on mice (two-year feeding experiment). Unpublished report No. 9344 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E. & Loeser, E. (1982) KWG 0519 (triadimenol, Baytan active ingredient). Chronic toxicological study on mice (feeding experiment over two years). Unpublished report No. 10855 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E. & Schilde, B. (1991) MEB 6447—chronic toxicity and cancerogenicity studies on Wistar rats with administration in diet over a period of 105 week. Unpublished report No. 20774 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Bomhard, E., Loeser, E. & Schilde, B. (1979) 1,2,4-triazole—subchronic toxicological study with rats (feeding study over three months). Unpublished report No. 8667 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Brendler, S. (1991) MEB 6447—mutagenicity test on unscheduled DNA synthesis in rat liver primary cell cultures in vitro. Unpublished report No. 20027 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Chopade, H.M. (1992) Disposition and metabolism of (phenyl-UL-14C)triadimefon in rats. Unpublished report No. 102640 from Miles Inc. Agriculture Division, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Cifone, M.A. (1982) Mutagenicity evaluation of KWG 0519 in the mouse lymphoma forward mutation assay. Final report. Unpublished report No. R2204 from Litton Biocinetics, Inc., Kensington, MD, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Clapp, M.J.L., Killick, M.E., Hollis, K.J. & Godley, M.J. (1983) Triazole alanine—teratogenicity study in the rat. Unpublished report No. CTL/P/875 from Imperial Chemical Industries PLC, Cheshire, England. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Clemens, G.R. & Hartnagel, R.E. (1990) Teratology study in the rabbit with MEB 6447 (triadimefon). Unpublished report No. BC5096 (MTD0149) from Toxicology Department Miles Inc., Elkhart, IN, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Clemens, G.R., Jasty, V., Troup, C.M. & Hartnagel, Jr, R.E. (1990a) A toxicity study in the rabbit with MEB 6447 (triadimefon). Unpublished report No. BC5106 (MTD0150) from Bayer CropScience AG. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Clemens, G.R., Troup, C.M. & Hartnagel, R.E. (1990b) Developmental toxicity study in the rat with Baytan technical. Unpublished report No. BC5142 from Miles Laboratories Inc., Elkhart, IN, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Clemens, G.R., Jasty, V., Troup, C.M. & Hartnagel, R.E. (1991) Developmental toxicity study in the rabbit with MEB 6447 (triadimefon). Unpublished report No. R5318 from Miles Laboratories Inc., Elkhart, IN, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Clemens, G.R., Grosso, D.S., Anderson, E.J. & Hartnagel, R.E. (1992) A developmental toxicity study in rabbits with Baytan technical. Unpublished report No. BC6692 from Miles Laboratories Inc., Elkhart, IN, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Crofton, K.M. (1996) A structure–activity relationship for the neurotoxicity of triazole fungicides. *Toxicol. Lett.*, **84**, 155–159.

Crofton, K.M., Boncek, V.M. & Reiter, L.W. (1988) Hyperactivity induced by triadimefon, a triazole fungicide. *Fundam. Appl. Toxicol.*, **10**, 459–465.

Deparade, E. (1986) CGA 131013 tech.—Salmonella/mammalian microsome mutagenicity test. Unpublished report No. 860187 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Deparade, E. (1984) CGA 142856—Salmonella/mammalian-microsome mutagenicity test. Unpublished report No. 840864 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Diesing, L. (1991) MEB 6447 (common name: triadimefon)—studies on skin sensitizing effect in guinea pigs (maximization test according to Magnusson and Kligman). Unpublished report No. 20867 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Dollenmeier, P. (1986) CGA 131013—point mutation test with Chinese hamster cells V79 (OECD conform). Unpublished report No. 860258 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Dreist, M. & Popp, A. (1996a) MEB 6447 (common name: triadimefon)—acute oral neurotoxicity screening study in Wistar rats. Unpublished report No. 24636 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Dreist, M. & Popp, A. (1996b) MEB 6447 (common name: triadimefon)—subchronic neurotoxicity screening study in Wistar rats (thirteen-week administration in the diet with a four-week recovery period in males and a ten-week recovery period in females). Unpublished report No. 25315 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Duah, F.K. & Smasal, T.M. (1993) Metabolism of triadimefon in laying hens. Unpublished report No. 105183 from Miles Inc. Agriculture Division, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Ecker, W. (1980) Biotransformation of 1,2,4-(3(5)-14C) triazole in rats. Unpublished report No. PF1471 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Eiben, R. (1984) MEB 6447 (triadimefon)—two-generation study with rats (supplementary study). Unpublished report No. 12712 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Flucke, W. (1979a) KWG 0519 form A—determination of acute toxicity (LD50): rat p.o. Unpublished report No. MO-00-010367 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Flucke, W. (1979b) KWG 0519 form B—determination of acute toxicity (LD50): rat. p.o. Unpublished report No. MO-00-010360 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Flucke, W. (1979c) KWG 0519 mixture 60 : 40—determination of acute toxicity (LD50). Unpublished report No. MO-00-010366 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Flucke, W. (1981) KWG 0519 (triadimenol)—study of sensitization effect on guinea pigs (maximization test of Magnusson & Kligman). Unpublished report No. 9894 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Frosch, I. (1998) Evaluation of skin sensitisation by 1,2,4-triazole with the guinea pig maximisation test. Unpublished report No. ToxLabs/1998/7050SEN from ToxLabs Prueflabor GmbH, Greppin, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hall, L.R. & Hartz, A.L. (1993) The metabolism of (14C) triadimefon in the lactating goat. Unpublished report No. 105161 from Miles Inc. Agriculture Division, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hamboeck, H. (1983a) Distribution, degradation and excretion of D,L-2-amino-3-(1-H-1,2,4-triazol-1-yl)propanoic acid (D,L-triazolylalanine) in the rat. Unpublished report No. 1/83 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hamboeck, H. (1983b) The metabolism of D,L-2-amino-3-(1H-1,2,4-triazol-1-yl)-propanoic acid (D,L-triazolylalanine) in the rat. Unpublished report No. 11/83 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Harbell, J.W. (1989) Bayleton technical—CHO/HGPRT mutation assay with confirmation. Unpublished report No. BC1143 from Microbiological Associates Inc., Rockville, MD, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Heimann, K.G. (1985a) KWG 1323 N (metabolite of KWG 0519)—study for acute oral toxicity to rats. Unpublished report No. 13618 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Heimann, K.G. (1985b) KWG 1342 N (metabolite of KWG 0519)—study for acute oral toxicity to rats. Unpublished report No. 13617 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Heimann, K.G. (1985c) KWG 1640 N (metabolite of KWG 0519)—study for acute oral toxicity to rats. Unpublished report No. 13616 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Heimann, K.G. & Schilde, B. (1984) KWG 0519 (suggested common name: triadimenol, the active ingredient of Baytan (TM))—subacute dermal toxicity study on rabbits. Unpublished report No. 12496 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Henderson, C. & Parkinson, G.R. (1981) R152056—acute oral toxicity to rats. Unpublished report No. CTL/P/600 from Imperial Chemical Industies Ltd, Cheshire, England. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1978a) KWG 0519—micronucleus test on mouse to evaluate KWG 0519 for potential mutagenic effects. Unpublished report No. 7588 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1978b) KWG 0519—dominant lethal study on male mouse to test for mutagenic effects (study No. 002). Unpublished report No. 7900 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1979a) KWG 0519—Salmonella/microsome test for detection of point-mutagenic effects (study No. KWG 0519/008). Unpublished report No. 8189 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1979b) KWG 0519—micronucleus test on mouse to evaluate KWG 0519 for potential mutagenic effects (study No. KWG 0519/009). Unpublished report No. 8584 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1981) KWG 0519 (triadimenol, the active ingredient of Baytan)—study of DNA damage using the *E. coli* Pol A1 test. Unpublished report No. 10265 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1982) THS 2212 triazolylanine—micronucleus test for mutagenic effect on mice. Unpublished report No. 11054 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1983a) THS 2212 triazolylalanine—Pol A1 test on *E. coli* during testing for effects harmful to DNA. Unpublished report No. 11390 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1983b) THS 2212 triazolylalanine—Salmonella/microsome test for point mutagenic effect. Unpublished report No. 11388 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1984) MEB 6447 (common name: triadimefon)—POL-test on *E. coli* to evaluate for potential DNA damage. Unpublished report No. 12780 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Herbold, B. (1986) MEB 6447 (common name: triadimefon)—cytogenetic study with human lymphocyte cultures in vitro to evaluate for harmful effect on chromosomes. Unpublished report No. 14982 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hertner, T. (1993) CGA 131013 technical—Salmonella and Escherichia/liver microsome test. Unpublished report No. 933002 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hill, D., Ikaiddi, M., Mazzio, E. & Soliman, K.F. (2000) The neurochemical basis for the behavioral effects of triadimefon. *Ann. NY Acad. Sci.*, **914**, 336–353.

Hoffmann, K. (1984) KWG 0519 (common name: triadimefon)—second chronic study of toxicity to dogs on oral administration (six-month feeding study). Unpublished report No. 12971 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hoffmann, K. & Groening, P. (1978) MEB 6447—long-term toxicity on dogs (two-year feeding study). Unpublished report No. 7882 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hoffmann, K. & Kaliner, G. (1977) KWG 0519—subchronic toxicity study on dogs (thirteen-week feeding experiment). Unpublished report No. 6633 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hoffmann, K. & Luckhaus, G. (1974) MEB 6447—subchronic toxicity study on dogs (thirteen-week feeding experiment). Unpublished report No. 5071 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Hoffmann, K. & Vogel, O. (1984) KWG 0519 (common name: triadimefon)—first chronic study of toxicity to dogs on oral administration (two-year feeding study). Unpublished report No. 12970 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Ikaiddi, M.U., Akunne, H.C. & Soliman, K.F. (1997) Behavioral and neurochemical effects of acute and repeated administration of triadimefon in the male rat. *Neurotoxicology*, **18**, 771–780.

Inukai, H. & Iyatomi, A. (1977) MEB 6447—mutagenicity test on bacterial systems. Unpublished report No. 68 from Nitokuno, Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Jagannath, D.R. (1980) Mutagenicity evaluation of MEB 6447 in the reverse mutation induction assay. Unpublished report No. R1675 from Litton Bionetics Inc., Kensington, Maryland, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Justus, K. (2002a) [Phenyl-UL-14C]triadimenol: distribution of the total radioactivity in male rats determined by quantitative whole body autoradiography. Unpublished report No. MR-091/02 from Bayer AG, Bayer CropScience, Monheim, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Justus, K. (2002b) [Phenyl-UL-14C]triadimenol: investigation of the biokinetic behaviour and the metabolism (ADME) in the rat. Unpublished report No. MR-088/02 from Bayer AG, Bayer CropScience, Monheim, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Kehrig, B. (1999) Occupational medical experiences with triadimenol. Unpublished report No. MO-99-017584 from Bayer AG, Dormagen, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Kehrig, B. & Steffens, W. (2003) Occupational medical experiences with triadimefon/Bayleton. Unpublished report No. MO-03-004770 from Bayer AG, Dormagen, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Keutz, E.V. & Groening, P. (1984) THS 2212 (triazolylalanine)—subchronic toxicity to dogs on oral administration (13-week feeding study). Unpublished report No. 12562 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Kimmerle, G. (1976) KWG 0519—subacute inhalation toxicity study on rats. Unpublished report No. 6321 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Kroetlinger, F. (1993) KWG 0519—study for skin and eye irritation/corrosion in rabbits. Unpublished report No. 22036 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Kroetlinger, F., Loeser, E., Schilde, B. & Diesing, L. (1982) KWG 0519 (triadimenol, the active ingredient of Baytan)—chronic toxicity study on rats (2-year feeding experiment). Unpublished report No. 11009 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Lai, K. & Simoneaux, B. (1986a) Balance study of ^{14}C-triazole acetic acid in orally dosed rats. Unpublished report No. ABR-86022 from Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Lai, K. & Simoneaux, B. (1986b) Balance study of ^{14}C-triazole alanine in orally dosed rats. Unpublished report No. ABR-86023 from Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Lai, K. & Simoneaux, B. (1986c) Balance study of ^{14}C-triazole in orally dosed rats. Unpublished report No. ABR-86021 from Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Lai, K. & Simoneaux, B. (1986d) The metabolism of ^{14}C-triazole acetic acid in the rat. Unpublished report No. ABR-86028 from Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Lai, K. & Simoneaux, B. (1986e) The metabolism of triazole alanine in the rat. Unpublished report No. ABR-86041 from Ciba-Geigy Corporation, Greensboro, NC, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Leeling, J.L., Helms, R.J. & Polomack, S.C. (1988) Dermal absorption of triadimenol-^{14}C in rats. Unpublished report No. 1055 from Miles Inc., Elkhart, Indiana, USA. Submitted to WHO for JMPR 1989 but not for the present Meeting.

Loeser, E. (1979) MEB 6447—multigeneration reproduction study on rats. Unpublished report No. 8297 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Loeser, E. & Eiben, R. (1982) KWG 0519—multigeneration study on rat. Unpublished report No. 11035 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Loeser, E. & Eiben, R. (1984) KWG 0519 (proposed common name: triadimenol)—generation study on rats. Unpublished report No. 12390 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Loeser, E. & Kaliner, G. (1977) KWG 0519—subchronic toxicity study on rats (three-month feeding experiment). Unpublished report No. 6811 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Machemer, L. (1976a) MEB 6447 (triadimefon)—evaluation for embryotoxic and teratogenic effects on rabbits following oral administration. Unpublished report No. 6297 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Machemer, L. (1976b) MEB 6447 (triadimefon)—evaluation for embryotoxic and teratogenic effects on rats following oral administration. Unpublished report No. 6294 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Machemer, L. (1976c) MEB 6447—dominant lethal study on male mice to test for mutagenic effects. Unpublished report No. 5837 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Machemer, L. (1977a) KWG 0519—evaluation for embryotoxic and teratogenic effects on orally dosed rats. Unpublished report No. 7038 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Machemer, L. (1977b) MEB 6447 (triadimefon, Bayleton active ingredient)—micronucleus test on mice to evaluate MEB 6447 for mutagenic effects. Unpublished report No. 6622 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Machemer, L. & Kimmerle, G. (1976) Evaluation of MEB 6447 (triadimefon) for embryotoxic and teratogenic effects on rats following inhalation in dynamic flow apparatus. Unpublished report No. 6298 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Maruhn, D. & Bomhard, E. (1984) Triazolylalanine (THS 2212)—study for subchronic toxicity to rats (three-month feeding study). Unpublished report No. 12397 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Melly, J.G. & Lohse, K. (1982) 1,2,4-Triazole—microbial mutagen test. Unpublished report No. 81R-252 from Rohm and Haas Company, Spring House, PA, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Menegola, E., Broccia, M.L., Di Renzo, F. & Giavini, E. (2001) Antifungal triazoles induce malformations in vitro. *Reprod. Toxicol*., **15**, 421–427.

Mihail, F. (1980) MEB 6447—acute toxicity studies. Unpublished report No. 9277 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Mihail, F. (1986) Triazolylanine (THS 2212). Unpublished report No. 11229A from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Mihail, F. & Kaliner, G. (1979) MEB 6447—subacute oral cumulative toxicity study on rats. Unpublished report No. 8195 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Mihail, F. & Thyssen, J. (1980) KWG 0519 (triadimenol)—acute toxicity studies (isomer ratio 80 : 20). Unpublished report No. 9451 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Mihail, F. & Vogel, O. (1981) KWG 0519 (triadimenol)—study of comparative toxicity to rats after 4-week treatment using two test compound samples. Unpublished report No. 9949 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Mihail, F. & Vogel, O. (1983) Triazolylanine (THS 2212)—subacute oral toxicity study on rats. Unpublished report No. 11491 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Milburn, G.M., Birtley, R.D.N., Pate, I., Hollis, K. & Moreland, S. (1986) Triazole alanine—two-generation reproduction study in the rat. Unpublished report No. CTL/P/1168 from Imperial Chemical Industries PLC, Cheshire, England. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Mohr, U. (1976) MEB 6447—subchronic toxicity study on rats (twelve-week feeding experiment). Unpublished report No. R840a from Medizinische Hochschule Hannover, Hannover, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Moser, V.C. & MacPhail, R.C. (1989) Neurobehavioral effect of triadimefon, a triazole fungicide, in male and female rats. *Neurotoxicol. Teratol*., **11**, 285–293.

Myhr, B.C. (1982) Evaluation of KWG 0519 in the primary rat hepatocyte unscheduled DNA synthesis assay—final report. Unpublished report No. R2212 from Litton Biocinetics, Inc., Kensington, MD, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Nagane, M., Hatanaka, J. & Iyatomi, A. (1982) KWG 0519—mutagenicity test on bacterial system. Unpublished report No. JAP220 from Nihon Tokusho Noyaku Seizo K.K., Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Nagashima, Y. (1982a) Primary eye irritation study of KWG 0519 in rabbits. Unpublished report No. BOZO/B-402 from Bozo Research Center Inc., Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Nagashima, Y. (1982b) Primary skin irritation study of KWG 0519 in rabbits. Unpublished report No. BOZO/B-401 from Bozo Research Center Inc., Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Nishimura, N. (1983) Subacute toxicity study of KWG0519 in dietary administration to rats for 13 weeks. Unpublished report No. BOZO/B-398 from Bozo Research Center Inc., Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Polacek, I. (1983a) Central nervous effects of KWG 0519 (pilot study). Unpublished report No. R2695 from Toxicological Institute Regensburg, Regensburg, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Polacek, I. (1983b) Safety pharmacology study with MEB 6447 on oral administration. Unpublished report No. R 2428 from Toxicological Institute, Regensburg, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Poth, A. (1989) *Salmonella typhimurium* reverse mutation assay with 1H-1,2,4-triazole. Unpublished report No. 158400 from CCR—Cytotest Cell Research GmbH & Co. KG, Rossdorf, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Procopio, K.R. & Hamilton, J.D. (1981) 1,2,4-Triazole—acute toxicity range-finding study. Unpublished report No. 81R-057A from Rohm and Haas Company, Spring House, PA, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Puhl, R.J. & Hurley, J.B. (1978) The metabolism and excretion of Baytan TM by rats. Unpublished report No. 66489 from Mobay Chemical Corporation, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Puri, E. (1986) CGA 131013 technical—autoradiographic DNA repair test on rat hepatocytes. Unpublished report No. 860184 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Putman, D.L. (1987) Baytan—sister chromatid exchange assay in chinese hamster ovary (CHO) cells. Unpublished report No. BC1022 from Microbiological Associates Inc., Rockville, MD, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Reeves, R., Thiruchelvam, M. & Cory-Slechta, D.A. (2004) Development of behavioral sensitization to the cocaine-like fungicide triadimefon is prevented by AMPA, NMDA, DA D1 but not DA D2 receptor antagonists. *Toxicol. Sci.*, **79**, 123–136.

Renhof, M. (1984) KWG 0519 (common name: triadimenol)—study for embryotoxic effects on rats after oral administration. Unpublished report No. 12687 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Renhof, M. (1988a) 1,2,4-triazole—investigations into embryotoxic effects on rats after oral administration—supplement to study No. T5019339. Unpublished report No. 17402 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Renhof, M. (1988b) 1,2,4-triazole—investigations into embryotoxic effects on rats after oral administration. Unpublished report No. 17401 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Richold, M., Allen, J.A., Williams, A. & Ransome, S.J. (1981) Cell transformation test for potential carcinogenicity of R152056. Unpublished report No. CTL/C/1085 from Huntingdon Research Centre Ltd., Huntingdon, England. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Roetz, R. (1982) MEB 6447—evaluation for embryotoxic effects on the rabbit after oral administration. Unpublished report No. 10831 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

San, R.H.C. & Springfield, K.A. (1990) Bayleton technical—Salmonella/mammalian microsome plate incorporation mutagenicity assay (Ames test). Unpublished report No. 5262 from Microbiological Associates, Inc., Rockville, MD, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Schladt, L. (1998) KWG 0519—oncogenicity study on CD-1 mice. Dietary administration over 18 months. Unpublished report No. 28299 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Schladt, L. & Sander, E. (1998) KWG 0519—subchronic toxicity study in CD-1 mice (administration in the feed over 13 weeks). Unpublished report No. 27070 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Schmidt, U. (1983) Triadimefon—interaction of triadimefon with liver microsomes—studies on rat and mouse in vivo and in vitro. Unpublished report No. 11812 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Sheets, L.P. (1990a) Dermal sensitization study with technical grade triadimefon (Bayleton) in guinea pigs. Unpublished report No. 5154 from Mobay Chemical Corporation, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Sheets, L.P. (1990b) Primary dermal irritation study with technical grade triadimefon (Bayleton) in rabbits. Unpublished report No. 5164 from Mobay Chemical Corporation, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Sheets, L.P. (1990c) Primary eye irritation study with technical grade Triadimefon (Bayleton) in rabbits. Unpublished report No. 5163 from Mobay Chemical Corporation, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Sheets, L.P. & Phillips, S.D. (1992) A repeated dose 21-day dermal toxicity study with technical grade triadimefon (Bayleton) in rats. Unpublished report No. BC6649 from Miles Inc. Agriculture Division, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Shirasu, Y., Moriya, M. & Miyazawa, T. (1978) Triadimefon—mutagenicity test on bacterial systems. Unpublished report No. 66747 (MO-02-005595) from Institute of Environmental Toxicology, Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Shirasu, Y., Moriya, M. & Miyazawa, T. (1979) Triadimefon—mutagenicity test on bacterial systems. Unpublished report No. 89028 (MO-02-004915) from Institute of Environmental Toxicology, Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Strasser, F. (1986) CGA 131013 technical—micronucleus test (Chinese hamster). Unpublished report No. 860185 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Tanahashi, N. & Moriya, M. (1982) Triadimenol—microbial mutagenicity study. Unpublished report No. MO-00-014878 from Institute of Environmental Toxicology, Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thevenaz, P. (1986) CGA 142856 technical—14-day subacute toxicity study in rats (dietary administration). Unpublished report No. 841140 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thevenaz, P. (1994) CGA 142856—acute oral LD50 in the rat. Unpublished report No. 840887 from Ciba-Geigy Limited, Basel, Switzerland. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thyssen, J. & Kaliner, G. (1976) KWG 0519—subacute oral cumulative toxicity study on rats (four-week treatment). Unpublished report No. 6481 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thyssen, J. & Kimmerle, G. (1974) MEB 6447—acute toxicity studies. Unpublished report No. 4416 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thyssen, J. & Kimmerle, G. (1976a) 1,2,4-Triazole—occupational toxicology study. Unpublished report No. 5926 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thyssen, J. & Kimmerle, G. (1976b) KWG 0519—acute toxicity studies. Unpublished report No. 6090 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thyssen, J. & Weischer, C.H. (1976) MEB 6447—subacute dermal cumulative toxicity study on rabbits. Unpublished report No. 6352 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Thyssen, J., Kimmerle, G. & Luckhaus, G. (1974) MEB 6447—subacute toxicity studies. Unpublished report No. 4464 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Unger, T.M., Goethem, Dv. & Shellenberger, T.E. (1982) A teratological evaluation of Bayleton in mated female rats. Unpublished report No. BC324 from Midwest Research Institute, Kansas City, MI, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

van Dijck, P. (1976) Evaluation of Bayleton (MEB 6447, triadimefon) for mutagenetic potential by the Ames test with histidine-auxotrophic *Salmonella typhimurium* strains. Unpublished report No. 53107 (MO-02-005632) from Laboratorium voor Hygiene, Katholieke Universiteit Leuven, Leuven, Belgium. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Vinggaard, A.M., Breinholt, V. & Larsen, J.C. (1999) Screening of selected pesticides for oestrogen receptor activation in vitro. *Food Addit. Contam.*, **16**, 533–542.

Vinggaard, A.M., Hnida, C., Breinholt, V. & Larsen, J.C. (2000) Screening of selected pesticides for inhibition of CYP19 aromatase activity in vitro. *Toxicol. In Vitro*, **14**, 227–234.

Walker, Q.D. & Mailman, R.B. (1996) Triadimefon and triadimenol: effects on monoamine uptake and release. *Toxicol. Appl. Pharmacol*., **139**, 227–233.

Walker, Q.D., Lewis, M.H., Crofton, K.M. & Mailman, R.B. (1990) Triadimefon, a triazole fungicide, induces stereotyped behavior and alters monoamine metabolism in rats. *Toxicol. Appl. Pharmacol*., **102**, 474–485.

Warren, D.L. (1990) Acute four-hour inhalation toxicity study with Bayleton technical in rats. Unpublished report No. 5267 from Mobay Chemical Corporation, Stilwell, KS, USA. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Watanabe, M. (1993) CGA-131013—DNA repair test (rec assay)—final report. Unpublished report No. IET 93-0010 from Kodaira, Tokyo, Japan. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Watkins, P.A. (1982) R152056: 3-(1,2,4-triazol-l-yl) alanine (ICI 156,342)—micronucleus test in CBC mice—TQM/4. Unpublished report No. CTL/C/1164 from Imperial Chemical Industries PLC, Cheshire, England. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Weber, H., Patzschke, K. & Wegner, L.A. (1978) 1,2,4-triazole-14C biokinetic studies on rats. Unpublished report No. PH7920 from Bayer AG, Wuppertal, Germany. Submitted to WHO by Bayer CropScience AG, Monheim, Germany.

Wickings, E.J., Middleton, M.C. & Hillier, S.G. (1987) Non-steroidal inhibition of granulosa cell aromatase activity in vitro. *J. Steroid Biochem*., **26**, 641–646.

Wickramaratne, G.A.D. (1987) The Chernoff-Kavlock Assay: Its validation and application in rats. *Teratog. Carcinog. Mutagen*., **7**, 73–83.

Zarn, J.A., Bruschweiler, B.J. & Schlatter, J.R. (2003) Azole fungicides affect mammalian steroidogenesis by inhibiting sterol 14 alpha-demethylase and aromatase. *Environ. Health Perspect*., **111**, 255–261.

TRIFLOXYSTROBIN

First draft prepared by
Ghazi Dannan[1] and Maria Tasheva[2]
[1] Office of Pesticide Programs, United States Environmental Protection Agency, Washington, DC, USA; and
[2] Laboratory of Toxicology, National Center of Hygiene, Medical Ecology and Nutrition, Sofia, Bulgaria

Explanation

Trifloxystrobin (methyl(*E*)-methoxyimino-{(*E*)-α-[1-(α,α,α,-trifluoro-*m*-tolyl)-ethylideneaminooxy]-*o*-tolyl}acetate) is a new broad-spectrum foliar fungicide that has not been evaluated previously by the JMPR.

Trifloxystrobin is being evaluated as a foliar fungicide for the control of fungi from the classes *Ascomycetes*, *Deuteromycetes*, *Basidiomycetes* and *Oomycetes*, in particular, for the treatment of powdery mildew and leaf spot diseases of pome fruit, grapes and bananas.

Trifloxystrobin is a synthetic derivative of the naturally occurring strobilurins found in several genera of wood-decaying fungi such as *Strobilurus tenacellus*. They have been shown to inhibit mitochondrial respiration by blocking electron transfer within the respiratory chain. As a consequence, important cellular biochemical processes are severely disrupted and fungal growth ceases. The intended fungicidal effects are derived from the parent molecule (CGA 279202) of the active ingredient, while the acid form is essentially inactive.

Evaluation for acceptable daily intake

1. Biochemical aspects

1.1 Absorption, distribution, and excretion

There are three studies of biokinetics and metabolism in rats dosed orally with radiolabelled trifloxystrobin. There are two studies of dermal absorption, a study of absorption in vitro in rat and human skin, and a study of dermal absorption in vivo in rats. Also, there are two studies of metabolism in goats given trifloxystrobin radiolabelled in each ring. The position of the ^{14}C radiolabel is shown in Figure 1.

The fate of [glyoxyl-phenyl-U-^{14}C]-labelled and [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin (radiochemical purity, >97%–>99%) was investigated in several groups of male and female rats after a single oral administration of ^{14}C-labelled compound at 0.5 or 100 mg/kg bw. An additional group received a single oral dose of [glyoxyl-phenyl-U-^{14}C]-labelled compound after 14 daily oral doses of unlabelled trifloxystrobin at 0.5 mg/kg bw. Urine, faeces, bile, and expired air were individually and separately collected. Blood was taken from three animals of each sex from each group by amputating the tip of the tail.

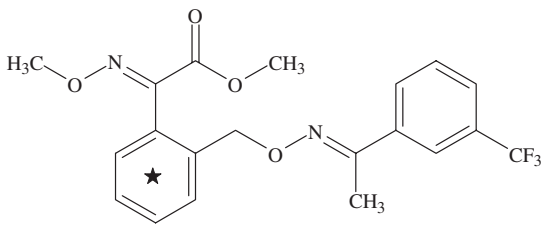
After administration of the trifloxystrobin at a dose of 0.5 mg/kg bw, 57% and 66% of the dose was absorbed (% of administered radiolabel present in urine + cage wash + bile + tissues) from the gastrointestinal tract in bile-duct cannulated male and female rats, respectively (Table 1). Absorption was slightly less after administration of trifloxystrobin at a dose of 100 mg/kg, based on decreased urinary and biliary elimination and a non-proportional increase of the area under the curve of concentration–time (AUC) (Tables 1 and 2).

Seven days after administration of [glyoxyl-phenyl-U-^{14}C]-labelled trifloxystrobin, 19% and 36% of the 0.5 mg/kg bw dose and 12% and 27% of the 100 mg/kg bw dose was excreted in the urine (including cage wash) of male and female rats, respectively (Table 1). The amount eliminated in the faeces was 79% and 63% at 0.5 mg/kg bw and 82% and 64% at 100 mg/kg bw in males and females, respectively (Table 1). Prior repeated dosing did not seem to significantly alter the pattern of excretion (Table 1).

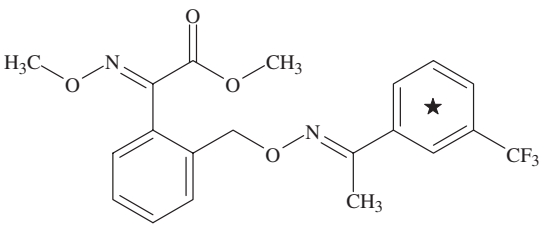
Experiments with bile-duct cannulated rats demonstrated that biliary excretion was the major route of elimination as 12%, 41%, 27% and 15%, 47% and 15% of the 0.5 mg/kg

Figure 1. Position of the ^{14}C radiolabel on trifloxystrobin used in studies of absorption, distribution and excretion

[Glyoxyl-phenyl-U-^{14}C]-trifloxystrobin



[Trifluormethyl-phenyl-U-^{14}C]-trifloxystrobin



★ = position of radiolabel

Table 1. Summary of data on excretion (% of administered dose) in rats given a single oral dose of ^{14}C-labelled trifloxystrobin

Radiolabel	[Glyoxyl-phenyl-U-^{14}C]										[Trifluormethyl-phenyl-U-^{14}C]	
Group	Lower dose		Repeated doses[a]		Higher dose		Bile-duct cannulated rats				Higher dose	
							Lower dose		Higher dose			
Sex	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	Female
Dose (mg/kg bw)	0.48	0.50	0.42	0.48	105.5	101.7	0.46	0.50	113.7	99.9	97.5	105.2
Urine:												
0–24 h	13.9	30.0	13.9	36.2	8.4	19.6	6.5	8.6	2.5	5.3	6.2	15.8
24–48 h	3.2	3.9	2.9	4.0	2.7	5.8	5.7	6.0	1.6	0.8[b]	2.4	8.8
48–168 h	1.7	1.3	1.6	1.5	1.0	1.2	—	—	—	—	1.0	2.2
Subtotal	18.8	35.2	18.4	41.7	12.1	26.6	12.2	14.6	4.1	6.1	9.6	26.8
Bile:												
0–48 h	—	—	—	—	—	—	41.0	46.5	34.7	19.1[c]	—	—
Faeces:												
0–24 h	57.1	50.3	54.5	41.0	45.9	32.6	7.6	5.4	8.6	20.8	52.7	25.8
24–48 h	19.0	11.5	20.1	11.8	33.2	26.9	19.7	9.2	35.6	8.3[b]	26.6	29.0
48–72 h	2.2	1.0	3.5	2.4	2.3	4.2	—	—	—	—	3.2	10.4
72–169 h	1.1	0.5	1.2	0.8	0.7	0.5	—	—	—	—	1.5	1.2
Subtotal	79.4	63.3	79.3	56.0	82.1	64.2	27.3	14.6	44.2	29.1	84.0	66.4
Expired air	—	—	—	—	<0.01	<0.01	—	—	—	—	0.08	0.05
Cage wash	0.3	0.5	0.1	0.3	0.2	0.4	1.0	0.9	0.3	0.4	0.4	0.7
Tissues	0.4	0.4	0.5	0.4	0.3	0.3	3.2	4.2	2.1	1.4	0.3	0.4
Total excretion	98.4	98.9	97.9	98.0	94.4	91.2	81.5	76.7	83.4	54.8	94.1	94.0

From Muller (1996)
[a] Daily oral doses (0.5 mg/kg) of unlabelled trifloxystrobin for 14 days
[b] 24–42 h
[c] 0–42 h

Table 2. Summary of blood kinetics in rats given radiolabelled trifloxystrobin as a single oral dose

Radiolabel	[Glyoxyl-phenyl-U-^{14}C]				[Trifluormethyl-phenyl-U-^{14}C]	
Group	Lower dose		Higher dose		Higher dose	
Sex	Male	Female	Male	Female	Male	Female
Dose (mg/kg bw)	0.48	0.49	105.0	99.4	96.5	105.5
C_{max} (ppm trifloxystrobin equivalents)	0.07	0.07	9.34	6.52	6.09	5.94
t_{cmax} (h)	12	12	24	12	24	12
$t_{cmax/2}$ (h)	48	23	50	44	67	52
AUC_{0-48h} (mg.h/kg)	2.7	1.6	334.6	214.3	229.7	214.8
AUC_{0-96h} (mg.h/kg)	3.8	2.3	—	—	375.1	331.6

From Muller (1996)
AUC, area under the curve

bw dose was excreted in the urine, bile and faeces of male and female rats, respectively (Table 1). The urinary and biliary excretion of bile-duct cannulated male and female rats was lower at 100 mg/kg bw than at 0.5 mg/kg bw. The decreased urinary excretion of [glyoxyl-phenyl-U-^{14}C]-labelled trifloxystrobin in bile-duct cannulated rats, especially in females, may be indicative of the involvement of an enterohepatic shunt mechanism in the elimination process. However, poor recoveries of excreted radiolabel may be responsible for the apparent decrease in urinary excretion (Table 1).

After administration of [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin at 100 mg/kg bw, elimination in the urine within 7 days was 10% in males and 27% in females, and in faeces was 84% in males and 66% in females, indicating that elimination of [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin was similar to that of [glyoxyl-phenyl-U-^{14}C]-labelled trifloxystrobin. The amount of the dose eliminated in expired air was insignificant and independent of the site of the radiolabel.

Maximum blood concentrations of residues were reached between 12 h and 24 h after a single oral administration, independent of the dose, the sex of the animals and the position of the radiolabel. The half-life ranged from 48–67 h and 23–52 h after dosing in male and female rats, respectively. The areas under the blood concentration–time curve (AUC) were increased by 129-fold at the 100 mg/kg bw when the dose was increased 200-fold, but were not influenced by the sex of the animals.

Assuming first-order kinetics, the half-lives of residues in all tissues ranged from 13 h to 33 h except for blood and spleen, which showed a retarded depletion of 30–82 h and 38–68 h, respectively (Table 3).

Seven days after a single oral dose of [glyoxyl-phenyl-U-^{14}C] trifloxystrobin at 0.5 mg/kg bw, the tissue concentration of residues did not exceed 0.014 ppm trifloxystrobin equivalents. Pre-treatment with unlabelled trifloxystrobin at a dose of 0.5 mg/kg bw for 14 consecutive days did not influence the pattern of distribution of tissue residues. At 100 mg/kg bw, the tissue concentrations of residues were 108–126 times higher than at the lower dose. Differences related to sex and label were apparent in the tissue residues (fat, kidneys, liver, and plasma). Concentrations of residues were generally higher in females than in males. The residues in the blood were associated predominantly with the blood cells and the extent of binding depended on the label and sex of the animals. The blood cell to plasma ratio was 4 : 1 and 18 : 1 for the [glyoxyl-phenyl-U-^{14}C] label and 11 : 1 and 17 : 1 for the [trifluormethyl-phenyl-U-^{14}C] label in male and female rats, respectively (Muller, 1996).

Table 3. Depletion of residual radioactivity (half-life [h]) from selected tissues in rats given [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin as a single oral dose

Tissue	Dose (mg/kg bw)			
	0.55	0.54	101.9	104.7
	Male	Female	Male	Female
Blood	38	30	40	82
Bone	30	13	26	28
Brain	15	27	31	33
Fat (abdominal)	18	18	18	33
Heart	23	19	26	26
Kidneys	23	21	31	30
Liver	21	15	28	23
Lungs	28	15	28	29
Muscle (skeletal)	20	18	24	25
Ovaries	NA	22	NA	24
Plasma	24	14	23	18
Spleen	39	38	42	68
Testes	26	NA	23	NA
Uterus	NA	22	NA	22

From Muller (1996)
NA, not applicable

In the earlier study, some label-related differences in the extent of tissue residues were observed at a dose of 100 mg/kg bw in rats. Therefore, another study was performed to investigate the disposition of [trifluormethyl-pheny-U-^{14}C]-trifloxystrobin (radiochemical purity, >98%), i.e. absorption, distribution and excretion at 0.5 mg/kg bw and the tissue depletion kinetics at 0.5 and 100 mg/kg bw. In addition, the experiment with bile-duct cannulated female rats dosed with [glyoxyl-phenyl-U-^{14}C]-trifloxystrobin (radiochemical purity, >99%) at 100 mg/kg bw was repeated because of inappropriate low recovery of radioactivity in the previous study. Urine, faeces, bile, and expired air were individually and separately collected. For the study of blood kinetics, blood was taken from three animals of each group and each sex by amputating the tip of the tail.

After administration of [trifluormethyl-phenyl-U-^{14}C]-trifloxystrobin at a dose of 0.5 mg/kg bw, the amount of radiolabel recovered in the urine and tissues within 7 days was twice as high in females (34%) as in males (17%). These data confirmed the findings of the first study.

After oral administration of a low dose of [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin, the radiolabel was rapidly and completely eliminated, predominantly in the faeces. Within 48 h, 93% of the administered dose was excreted in males and females, and the administered dose was completely eliminated within 7 days after administration. The route of elimination was influenced by the sex of the animals: female rats excreted twice as much radiolabel in the urine (33% of the administered dose) as the males (16%). The faeces contained 80% and 62% of the administered dose in male and female rats, respectively. These data confirmed the findings of the first study.

After a single oral administration of [trifluormethyl-phenyl-U-^{14}C]-trifloxystrobin at a dose of 0.5 mg/kg bw, two blood concentration maxima were observed, at approximately 0.5 h and 12 h. In females, the first maximum concentration exceeded the second, while in males the second exceeded the first. Thereafter, the amount of radioactivity in the blood depleted at a moderate rate. Assuming monophasic first-order kinetics, a half-life of 40 h was determined that was independent of the sex of the animals. The areas under the blood concentration–time curve (AUC_{0-96h}) were in the same range for male and female rats, indicating a similar bioavailability. These data confirmed the findings of the first study.

Independent of the dose administered or the sex of the animal, the highest tissue concentrations of residues were generally found between 12 h and 24 h after administration. The terminal concentrations of residues were very low at 7 days after a single oral administration of [trifluormethyl-phenyl-U-^{14}C]-trifloxystrobin at 0.5 mg/kg bw, irrespective of the sex of the animal. The highest concentrations of radiolabel were found in the blood, kidneys and liver amounting to 0.014/0.009 ppm (male/female), 0.010/0.012 mg/kg, and 0.012/0.007 mg/kg, respectively. All the other tissue concentrations of residues did not exceed 0.006 mg/kg. No significant differences were found between this data and that for the first study using the glyoxyl-phenyl radiolabel.

The residual radioactivity was depleted from tissues and organs with a half-life of 12–37 h independent of the dose and sex of the animals, except for blood (25–41 h) and spleen (22–99 h). These data confirmed the findings of the first study.

On the basis of urinary and biliary excretion and the radioactivity retained in the tissues of female rats dosed with [glyoxyl-phenyl-U-^{14}C]-trifloxystrobin at 100 mg/kg bw,

approximately 22% of the administered dose was absorbed into the systemic circulation. Within 48 h, the bile-duct cannulated female rats excreted approximately 18%, 3% and 20% in the bile, urine and faeces, respectively. The sponsor stated that the low recovery determined in the previous study was caused by the incorrect determination of the dose remaining in the gastrointestinal tract (Stampf, 1998).

Dermal absorption of trifloxystrobin was investigated in vitro in isolated rat and human epidermis. A study of absorption in vitro compared the dermal absorption of trifloxystrobin, as a 125 EC formulation, in human and rat skin. The blank formulation (purity, 88.3%) was mixed with 11.7% (w/w) radiolabelled (radiochemical purity, >95%) or unlabelled (purity, 99.9%) trifloxystrobin at three different concentrations. The resulting mixtures were either applied undiluted (10.26 mg/cm^2) or after dilution with water (1.478 and 0.236 mg/cm^2). Composition of the blank formulation is shown in Table 4.

Epidermal membranes of human skin from a female Caucasian donor aged 43 years were prepared immediately before the start of the study by immersion of the pre-frozen skin in water heated to approximately 60 °C. Skin was also taken from male Sprague-Dawley rats aged 26 days. Epidermal membranes were prepared immediately before the start of the study by overnight immersion in 2 mol/l sodium bromide, containing 0.01% sodium azide. Sterile glass rings (internal area, approximately 0.64 cm^2) were glued onto the epidermis. The epidermis was then transferred onto a Netwell insert (200 µm mesh) in a six-well plate, allowing contact of the basal membrane with the receptor fluid, while the stratum corneum remained exposed to air. In all test groups, 50 µl of test solutions were applied into the glass rings. [4-^{14}C]-Labelled testosterone was used as a reference compound. Final concentrations of trifloxystrobin and applied doses in the three samples are shown in Table 5.

The concentration of trifloxystrobin at the higher dose corresponds to that of the undiluted product, while the concentration at the lower dose corresponds to that of a typical

Table 4. Composition of blank formulation used in a study of dermal penetration in vitro

Component	Proportion (% w/w)
Copolymer butanol 34 PO/22 EO	11.7
Tristyrylphenol 16 EO	9.3
Styrylphenol polyethoxyester phosphate	2.3
1-Methyl-2-pyrrolidone	65.0

From Van de Sandt (1997)

Table 5. Dose and concentration of ^{14}C-labelled trifloxystrobin applied to rat or human skin membranes in a study of dermal penetration in vitro

Group	Concentration		Dose (mg/cm^2)
	mg/ml	MBq/ml	
A1 (low)	3.023	0.31	0.236
A2 (intermediate)	18.924	0.32	1.478
A3 (high)	131.388	0.34	10.265
B (testosterone)	0.299	0.74	0.015

From Van de Sandt (1997)

spray solution of the respective formulation. Samples of receptor fluid (200 µl) were collected at 1-h intervals for the first 12 h, then at 2-h intervals until 24 h after application. Thereafter, samples were collected at 4-h intervals until the end of the study (48 h). Test compound remaining was removed from the membrane with cotton swabs soaked in ethanol, and epidermal membranes were solubilized in 1.5 mol/l potassium hydroxide and 20% ethanol. The remaining receptor fluid was collected and wells were washed with ethanol.

The absorption of trifloxystrobin was non-linear and was faster in the rat epidermis than in human epidermis (see Tables 6 and 7). The penetration of testosterone was similar to that described in the data on historical controls for the laboratory, according to the sponsor (Van de Sandt, 1997).

In a study of absorption in vivo, trifloxystrobin, as a 125 EC formulation, was applied to the shaved backs of male rats aged 8 weeks at a higher (1.12–1.14 mg/cm^2) or lower (0.024–0.026 mg/cm^2) dose to replicate exposure to either diluted or concentrated product. The blank formulation (purity, 88.3%) was identical to that described above (Table 4) in the study of dermal penetration in vitro. The blank formulation was mixed with either: (a) 11.7% (w/w) of radiolabelled trifloxystrobin (purity, >95%) for the lower dose; or (b) a mixture of radiolabelled and unlabelled trifloxystrobin (purity, >99.9%) for the higher dose. The resulting mixtures were either applied undiluted (higher dose) or after dilution with water (lower dose). The final concentrations of trifloxystrobin and radiolabel, and applied doses for the two dosing solutions are shown in Table 8.

Table 6. In-vitro percutaneous absorption of trifloxystrobin in rat epidermis

	Dose (mg/cm^2)		
	0.236	1.478	10.265
Penetration (% of dose (µg/cm^2)):			
Within 8 h	0.58 (1.37)	0.50 (7.32)	0.43 (44.50)
Within 24 h	1.66 (3.93)	1.35 (19.93)	0.72 (74.19)
Within 48 h	3.52 (8.32)	2.18 (32.25)	1.24 (127.69)
Flux constant (µg/cm^2 per h)	0.28	1.35	13.88
K_p value (cm/h.10^{-5})	9.20	7.14	10.56
Lag time (h)	3.6	4.0	0.2

From Van de Sandt (1997)

Table 7. In-vitro percutaneous absorption of trifloxystrobin in human epidermis

	Dose (mg/cm^2)		
	0.236	1.478	10.265
Penetration (% of dose (µg/cm^2)):			
Within 8 h	0.06 (0.15)	0.03 (0.51)	0.05 (4.72)
Within 24 h	0.26 (0.62)	0.11 (1.56)	0.13 (13.56)
Within 48 h	0.61 (1.44)	0.23 (3.35)	0.26 (26.43)
Flux constant (µg/cm^2 per h)	0.03	0.07	0.77
Kp value (cm/h.10^{-5})	1.09	0.38	0.59
Lag time (h)	5.6	1.6	2.2

From Van de Sandt (1997)

Table 8. Dose and concentration of ^{14}C-labelled trifloxystrobin applied to male rat skin in a study of absorption in vivo

Group	Concentration		Dose (mg/cm^2)
	mg/g	MBq/g	
Lower dose	3.30	2.90	0.024–0.026
Higher dose	116.93	4.23	1.12–1.14

From De Bie (1997)

Table 9. Blood kinetics of radiolabel after dermal application of ^{14}C-labelled trifloxystrobin in male rats

Time-point (h)	Concentration (µg parent compound equivalents/g blood)	
	Lower dose—24 µg/cm^2	Higher dose—130 µg/cm^2
0.5	0.009	0.07
1	0.011	0.10
2	0.007	0.13
4	0.012	0.10
6	0.009	0.09
8	0.008	0.12
12	0.011	1.65
24	0.013	0.30
48	0.013	0.39

From De Bie (1997)

Table 10. Summary of dermal absorption (expressed as % of applied dose) of trifloxystrobin in male rats

Sample	Lower dose (24 µg/cm^2)			Higher dose (1130 µg/cm^2)		
	8 h	24 h	48 h	8 h	24 h	48 h
Urine (total)	0.18	0.51	1.43	0.09	0.51	1.43
Faeces (total)	0.01	3.34	9.47	0.01	1.06	3.49
Cage wash	0.04	0.20	0.46	0.01	0.09	0.36
Control skin + blood	0.49	0.07	0.13	0.18	0.13	0.16
Carcass	4.39	3.73	4.81	9.85	4.19	4.54
Absorbed	5.11	7.85	16.30	10.14	5.70	9.25
Application site	28.11	19.05	21.57	12.18	7.08	5.58
Not absorbed	61.92	67.67	75.72	78.86	89.06	85.80

From De Bie (1997)

Concentrations of radiolabel found in the blood were generally low, close to the limit of determination. For the lower dose (0.024–0.026 mg/cm^2), the concentration remained constant throughout the entire observation period. For the higher dose (1.12–1.14 mg/cm^2), the highest concentration was obtained at 12 h, and was most probably induced by the washing procedure, according to the sponsor (Table 9).

Trifloxystrobin was absorbed to a moderate extent by rat skin. After 8 h and 24 h, 5–10% of the applied dose was absorbed, independent of the dose level (Table 10). It should be noted that in animals given the higher dose, the absorption value of 10.14% at 8 h is too high and is likely to be inaccurate because of the time-course trends in absorption at the lower and higher doses. In rats given the lower dose, 28% of the applied dose remained in the skin after washing. Depletion of this radioactivity was slow, dropping to approximately 20% after 48 h (Table 10). A similar pattern was observed in animals given the higher dose;

about 12% remained in the skin after washing and this amount decreased to about 5% after 48 h. The rate of dermal absorption over 48 h was considered to be 16% on the basis of continued absorption of trifloxystrobin following the 8 h wash and removal from the application site in rats given the lower dose (De Bie, 1997).

1.2 Metabolism

Specimens from the main study of toxicokinetics (Muller, 1996) and the supplementary study of toxicokinetics (Stampf, 1998) were analysed to determine the metabolic pathway of trifloxystrobin in male and female rats. Radioactivity in urine and other liquid specimens was measured by liquid scintillation counting (LSC). The radioactivity in aliquots of faeces and other solid specimens was determined after combustion. Fractions of extracted specimens were separated and analysed by thin-layer chromatography (TLC) and high-performance liquid chromatography (HPLC). The pattern of radioactivity on TLC plates was detected with a spark chamber camera or a biomaging analyser and quantified by scraping off the radioactive fractions and analysing by LSC. Non-radioactive fractions on TLC plates were located under ultraviolet light at 254 nm. Extracts of urine, bile, and faeces were pre-purified by solid-phase extraction (SPE). Mass spectrometry (MS) and nuclear magnetic resonance (NMR)–spectroscopy as well as high-voltage electrophoresis were used to elucidate the structures of metabolites. In addition, CGA 347242 and CGA 373463 were characterized as metabolites by comparison with authentic reference substances.

The patterns of metabolites in the urine were complex; they were qualitatively independent of pretreatment and slightly dependent on dose, but showed significant differences dependent on sex and position of the radiolabel. In total, there were about 26 urinary metabolite fractions, most of which represented about 1% or less of the administered radiolabel in addition to an unresolved fraction that comprised 9–40% of the administered radiolabel. In the radioactivity extracted from the faeces, there were about 10 resolved metabolite fractions whose patterns were qualitatively independent of sex, dose, pretreatment, and position of radiolabel, with some quantitative variations. In addition, some of the faecal-extracted radiolabel was unresolved (9–24% of the administered dose) while the non-extractable fraction represented 5–15% of the administered radiolabel. Also, the patterns of metabolites in the bile were complex and qualitatively independent of sex and dose, with some quantitative variations. The patterns in the urine, faeces, and bile were essentially distinct from each other.

Thirty-five metabolites were isolated from urine, faeces, and bile of the male and female rats at the highest dose and were identified by spectroscopy. In addition, CGA 347242, CGA 373463, NOA 414412, and NOA 417076 were characterized as metabolites by co-chromatography with authentic reference substances. Some of the metabolites (mainly urinary) were unique to one of the two sites of the radiolabel indicating cleavage between the glyoxyl-phenyl and trifluoromethyl-phenyl moieties. The proposed metabolic pathway is shown in Appendix 1.

On the basis of the structures of the metabolites, the following metabolic pathways for trifloxystrobin were derived:

— Hydrolysis of the methyl ester to the corresponding acid (e.g. CGA 321113) (major pathway);

— *O*-Demethylation of the methoxyimino group yielding a hydroxyimino compound (e.g. NOA 405637) (major pathway);
— Oxidation of the methyl side-chain to a primary alcohol (e.g. Met 2U), followed by partial oxidation to the respective carboxylic acid (e.g. Met 13U) (major pathway);
— Hydrolysis of the imino group of the glyoxyl-phenyl moiety to yield a ketone, with subsequent chain shortening by oxidative decarboxylation ultimately producing a benzoic acid derivative (minor pathway);
— Chain shortening of the glyoxyl moiety by oxidative decarboxylation, giving rise to a benzoic acid amide (minor pathway);
— Hydroxylation of the phenyl rings (minor pathways);
— Oxidation of the hydroxyimino group to produce a nitro group (minor pathway);
— Cleavage between the glyoxyl-phenyl and trifluoromethyl-phenyl moiety

Cleavage between the glyoxyl-phenyl and trifluoromethyl-phenyl moiety accounted for about 10% of the applied dose. The primary cleavage products were prone to further degradation mainly by the above mentioned processes. For the trifluoromethyl-phenyl part, these included oxidation of the hydroxyimino group leading to a nitro compound, oxidation of the methyl group resulting ultimately in a carboxylic acid, hydrolysis of the imino group producing a ketone, followed by oxidation of the methyl group to an intermediary carboxylic acid. This α-keto acid can either be reduced to an α-hydroxy acid or may undergo chain shortening by oxidative decarboxylation to trifluoromethyl-benzoic acid. The other fragment (glyoxyl-phenyl part) is transformed by oxidation of the benzylic substituent to a benzoic acid. *O*-Demethylation of the methoxyimino group yielding a hydroxyimino compound, hydrolysis of the imino group to an α-keto acid and subsequent chain shortening by oxidative decarboxylation ultimately yields phthalic acid.

Glucuronic and, to a lesser extent, sulfuric acid conjugates were generated from metabolites containing a hydroxy group. The majority of metabolites resulted from more than one of the above mentioned transformations. Approximately 4–7% and 31–47% of the lower and higher dose, respectively, was eliminated in the faeces as unchanged trifloxystrobin. The oxidation of the methyl side-chain to a primary alcohol was more pronounced in female rats, resulting in sex-specific major metabolites mainly in the urine. The degradation resulted in metabolites that were eliminated at a moderate rate. The absorbed portion of the administered dose was almost completely degraded and eliminated mainly via the bile and to a lesser extent via urine. Bile metabolites were mostly glucuronic and tentatively sulfuric acid conjugates. After hydrolysis by the gut microflora, these metabolites were ultimately eliminated via faeces, together with unchanged trifloxystrobin escaping absorption, or via urine after enterohepatic circulation and further transformation (Thanei, 1997).

Two studies were carried out in lactating goats to investigate the metabolic fate of [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin or [glyoxyl-phenyl-U-^{14}C]-labelled trifloxystrobin.

In the first study, two lactating female goats received gelatin capsules containing [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin (radiochemical purity, >99%) at doses equivalent to 103.8 mg/kg feed or 4.24 mg/kg bw on 4 consecutive days. Milk, urine, faeces, bile, and cage wash were collected. The animals were sacrificed 6 h after the last dose. Blood, muscle, fat, liver, and kidney were taken from both animals.

During the in-life period, 0.08%, 44.5% and 17.4% of the total dose were eliminated via milk, faeces and urine, respectively. Tissue concentrations of residues were: leg muscle, 58 ppb trifloxystrobin equivalents; tenderloin, 59 ppb; omental fat, 182 ppb; perirenal fat, 209 ppb; liver, 4815 ppb; and kidneys, 1830 ppb. Tissue concentrations of residues in milk were 85 ppb (interval 0–78 h), and blood and bile contained 248 ppb and 71 315 ppb, respectively. The extractability of the milk and tissue samples was good (>90%) with the exception of liver (66.5%) where microwave-assisted extraction was used to release additional radioactive residues thereby increasing the extractability to 95.0%.

Trifloxystrobin was found to be the major compound in fat (79.0%) and in milk (51.6%) and to a smaller extent in muscle (20.6%), liver (2.8%), kidneys (1.8%) and faeces (21.7%). Hydrolysis of trifloxystrobin produced CGA 321113, i.e. methoxyimino-{2-[1-(3-trifluoromethy-phenyl)-ethylidene-aminooxymethyl]-phenyl}-acetic acid, being the major metabolite excreted via urine (70.4% of the urine radioactivity) and faeces (35.5%). This hydrolysis product was also the major metabolite in muscle (57.2%) and kidneys (54.3%) and was also found in all other samples, i.e. fat (10.4%), liver (20.0%) and milk (3.6%). The major metabolite in liver (27.8%) was metabolite L7a, i.e. the taurine conjugate of CGA 321113 also present in muscle (1.2%), kidneys (12.7%) and milk (13.0%). Metabolite L7b was identified as the glycine conjugate of CGA 321113. This conjugate was found in muscle (1.2%), liver (10.7%), kidneys (5.2%) and faeces (2.9%).

Demethylation of the methoxyimino group of trifloxystrobin to metabolite 2F, i.e. hydroxyimino-(2-[1-(3-trifluoromethyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-acetic acid methyl ester was only found in faeces (10.2%). Metabolite 1U, the demethylation product of CGA 321113, i.e. hydroxyimino-{2-[1-(3-trifluoromethyl-phenyl)-ethylidene-aminooxymethyl]-phenyl}-acetic acid, was identified in muscle (1.3%), fat (0.5%), kidneys (1.9%), milk (0.9%), urine (7.2%) and faeces (3.0%).

Hydroxylation of the aminooxymethyl group was observed in the monohydroxylated metabolite 2U, i.e. {2-[2-hydroxy-l-(3-trifluoromethyl-phenyl)-ethylidene-aminooxymethyl]phenyl}-methoxyimino-acetic acid in muscle (2.0%), fat (0.4%), kidneys (3.1%), milk (1.9%), urine (3.2%), faeces (4.1%) and in the dihydroxylated metabolite 6U, i.e. hydroxyimino-{2-[2-hydroxy-1-(3-trifluoromethyl-phenyl)-ethylidene-aminooxymethyl]phenyl)-acetic acid in liver (4.4%) and milk (4.0%).

Hydroxylation of the glyoxyl-phenyl ring in position four was found in metabolite 7F, i.e. {4hydroxy-2-[1-(3-trifluoromethyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-methoxyiminoacetic acid methyl ester. This metabolite was only characterized in faeces (8.0%).

Cleavage of the molecule between the two phenyl rings was a minor pathway, as shown by the presence of metabolite 12 U, i.e. hydroxy-(3-trifluoromethyl-phenyl)-acetic acid in muscle (1.7%), fat (0.7%), milk (3.1%) and urine (3.7%) and metabolite 11 U, i.e. sulfuric acid mono-[1-(3-trifluoromethyl-phenyl)-ethanone oxime]ester in kidneys (0.3%) and urine (4.5%) (Rumbeli, 1997a).

In the second study, two lactating female goats received gelatin capsules containing [glyoxyl-phenyl-U-^{14}C]-labelled trifloxystrobin (radiochemical purity, >98%) at daily doses equivalent to 100.4 mg/kg feed or a dose of 4.13 mg/kg bw on 4 consecutive days. The animals were sacrificed 6 h after the last dose.

During the study, 0.06%, 36.0% and 18.9% of the total administered dose was eliminated via milk, faeces, and urine, respectively. Tissue concentrations of residues were: leg muscle, 77 ppb trifloxystrobin equivalents; tenderloin, 74 ppb; omental fat, 364 ppb; perirenal fat, 343 ppb; liver, 3913 ppb; and kidneys, 2331 ppb. Milk contained 89 ppb (interval 0–78 h) and blood and bile contained 330 ppb and 40 813 ppb, respectively. The extractability of the milk and tissue samples was good with the exception of liver (68.7%) where microwave-assisted extraction was used to release additional radioactive residues, thereby increasing the extractability to 97.9%.

Trifloxystrobin was found to be the major compound in fat (82.0%) and in milk (73.8%) and to a smaller extent in muscle (26.5%), liver (2.5%) and kidneys (1.8%). Trifloxystrobin was the major residue eliminated via the faeces (48.2%). Findings on metabolites, tissue distribution, and residue concentrations were very similar to those described earlier for [trifluormethyl-phenyl-U-^{14}C]-labelled trifloxystrobin.

In this study there was not a significant amount of label-specific metabolites, i.e. cleavage of the molecule between the two phenyl rings did not seem to be a major reaction in the metabolism of trifloxystrobin. In conclusion, the metabolism of trifloxystrobin in the goat follows the same major pathways as in the rat (Figure 2) (Rumbeli, 1997b).

2. Toxicological studies

2.1 Acute toxicity

The results of studies of acute toxicity with trifloxystrobin, performed in compliance with OECD guidelines and good laboratory practice (GLP), are summarized in Table 11.

Trifloxystrobin has low acute oral toxicity in rats and mice (LD_{50} > 5000 mg/kg), low acute dermal toxicity in rats and rabbits (LD_{50} > 2000 mg/kg), low acute inhalational toxicity in rats (LC_{50} > 4650 mg/m^3), slight skin irritancy in rabbits, is a moderate eye irritant in unwashed rabbit eyes but a non-irritant in washed rabbit eyes, and is a skin sensitizer in guinea-pigs by the maximization test but not by the Buehler test.

Table 11. Acute toxicity of trifloxystrobin

Species	Strain	Sex	Route	LD_{50} (mg/kg bw)	LC_{50} (mg/l air)	Reference
Mouse	Tif: MAG	Male & female	Oral	>5000	—	Winkler (1996)
Rat	Crl: CD (SD)	Male & female	Oral	>5000	—	Glaza (1994a)
Rat	HSD: (SD)	Male & female	Inhalation	—	>4.65	Holbert (1995)
Rat	Tif: RAI f (SD)	Male & female	Dermal	>000	—	Marty (1995)
Rabbit	Hra: (NZW)	Male & female	Dermal	>2000	—	Glaza (1994b)
Rabbit	Hra: (NZW)	Male & female	Skin irritation	Slightly irritating	—	Glaza (1994c)
Rabbit	Hra: (NZW)	Male & female	Eye irritation	Moderately irritating (unwashed)	—	Glaza (1994d)
		Female		Non-irritating (washed)		
Guinea-pig	Tif: DHP (White)	Male & female	Skin sensitization: maximization	Sensitizing	—	Marty (1994)
	Crl: (HA)BR	Male	Skin sensitization: Buehler	Non-sensitizing	—	Glaza (1994e)

Figure 2. Proposed metabolism of trifloxystrobin (CGA 279202) in rats, goats and hens

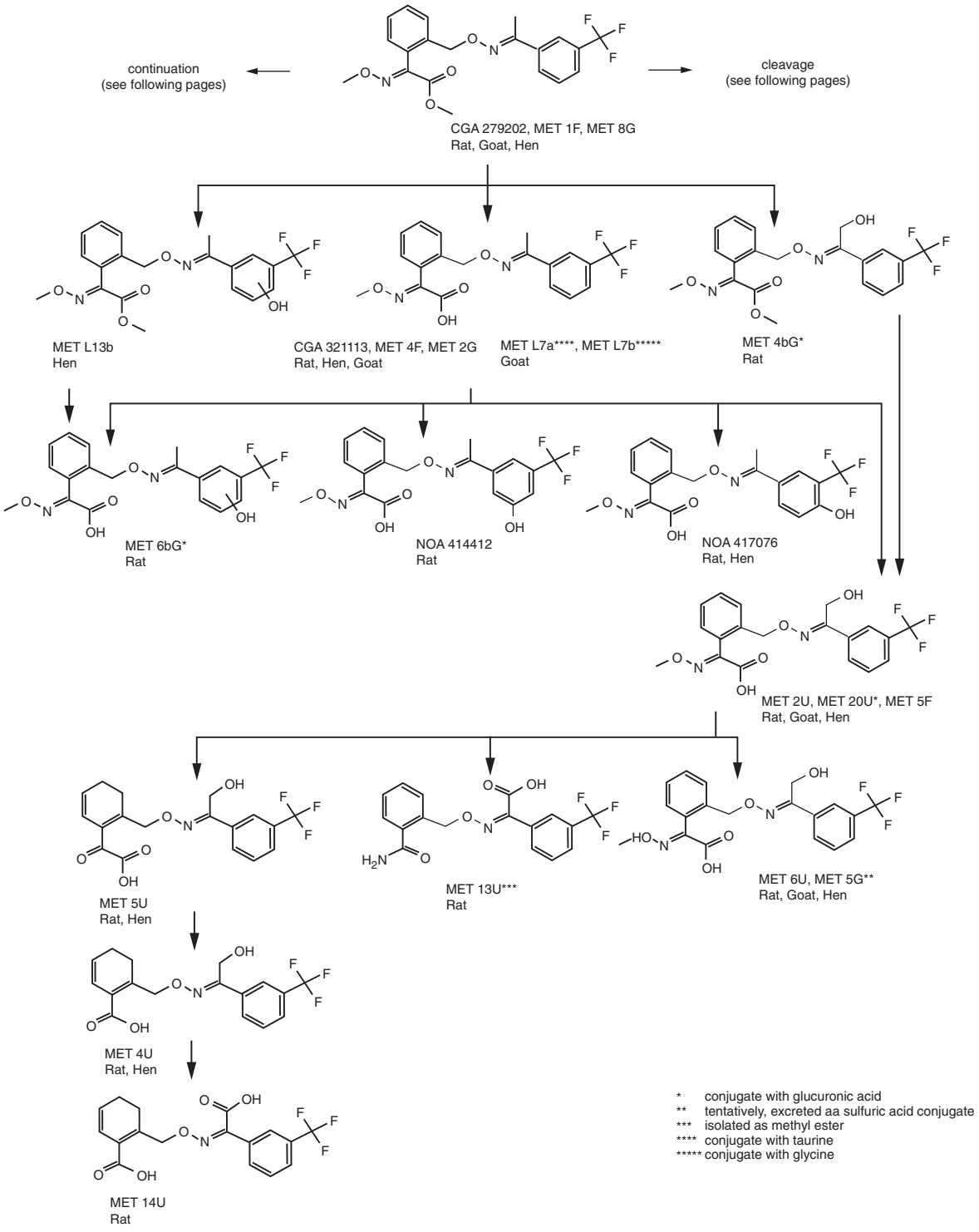


Figure 2. Continued

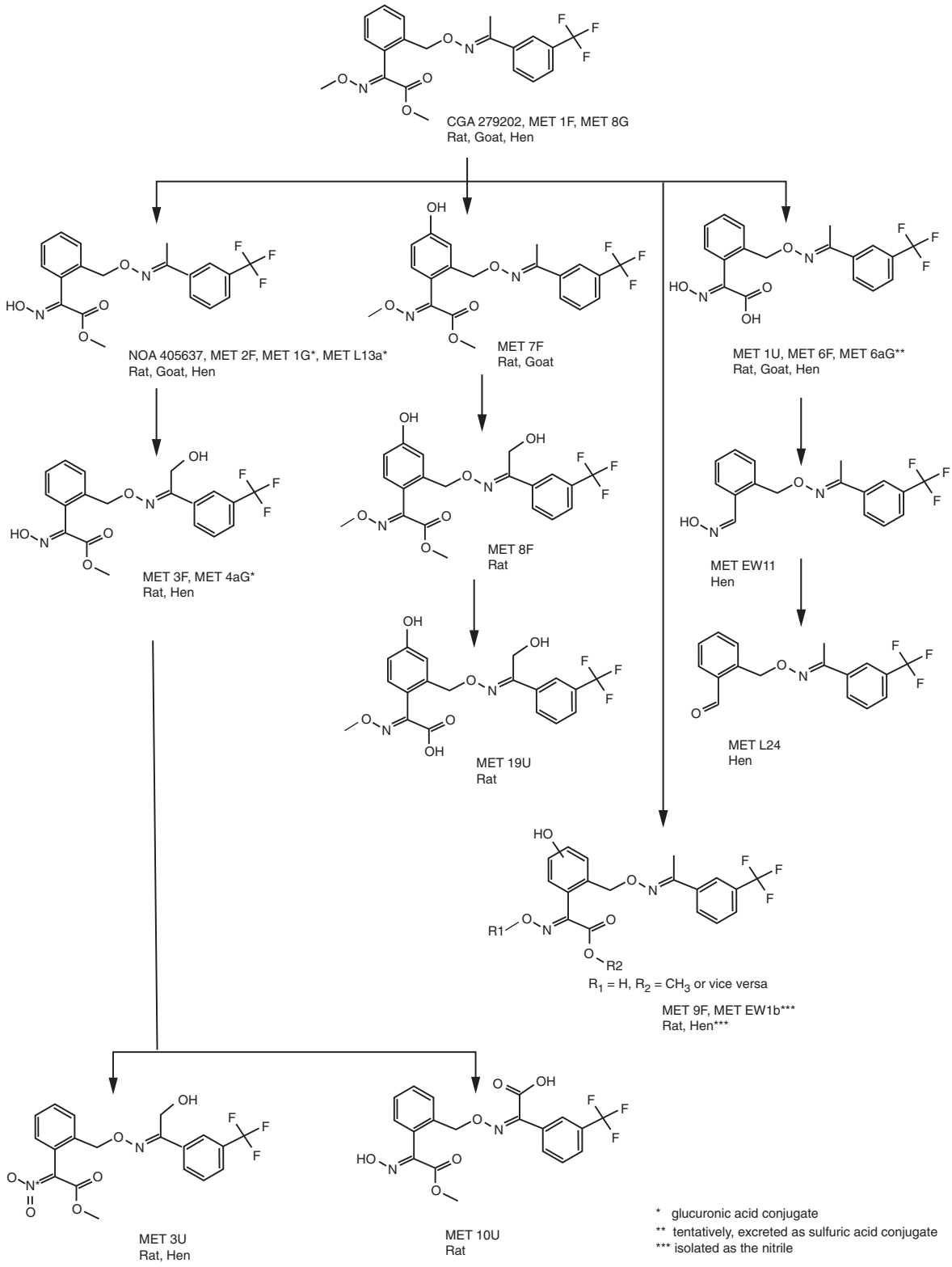
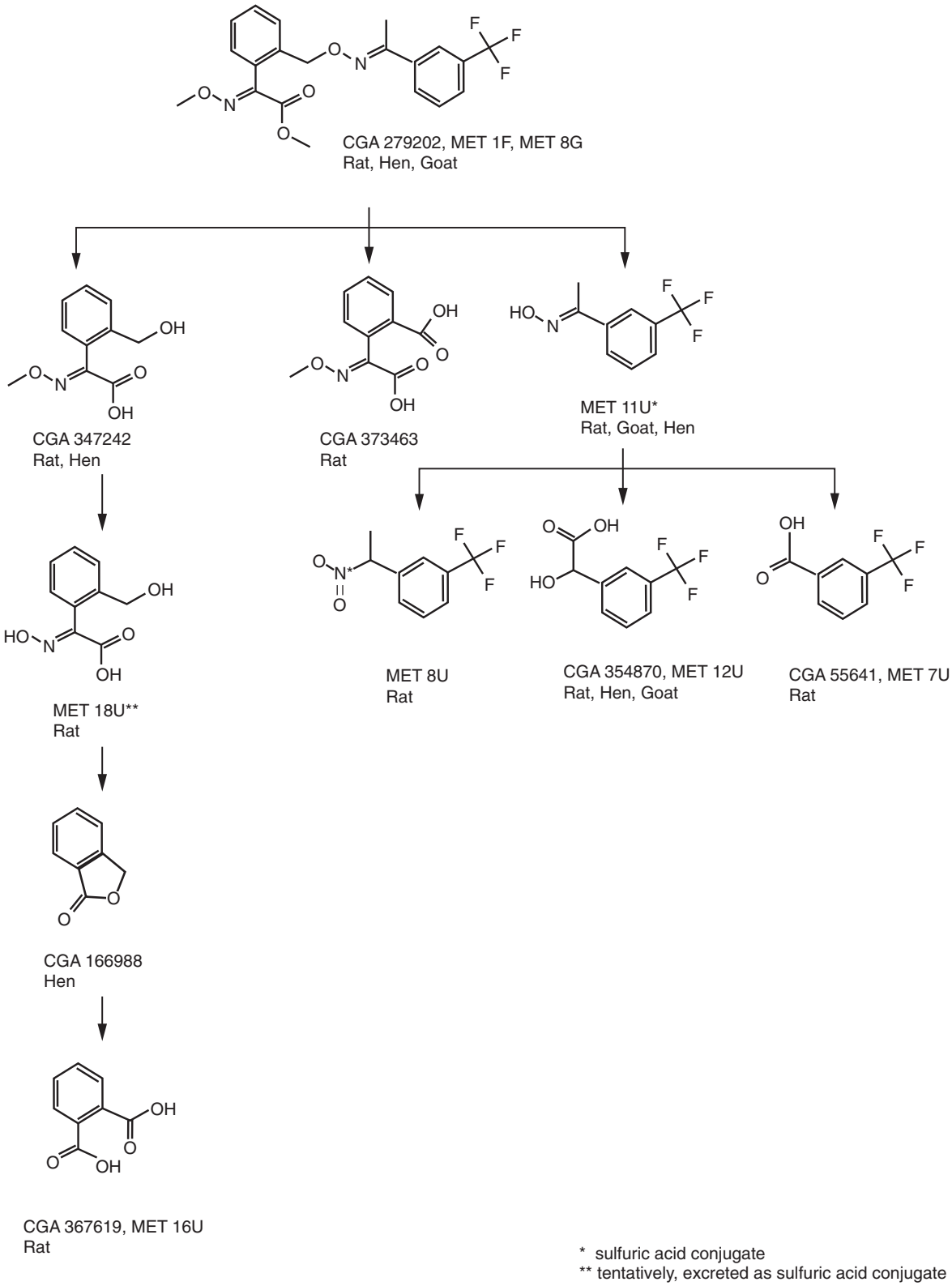


Figure 2. Continued



* sulfuric acid conjugate
** tentatively, excreted as sulfuric acid conjugate

2.2 Short-term studies of toxicity

Mice

In a study conducted in compliance with the principles of GLP (with QA certification), groups of 10 male and 10 female mice (Tif: MAGf) were continuously fed with diets containing trifloxystrobin (purity, 96.2%) at a concentration of 0, 500, 2000 or 7000 ppm (equal to 0, 76.9, 315.1 and 1275 mg/kg bw per day in males and 0, 110.4, 425.1 and 1649 mg/kg bw per day in females, respectively) for 3 months. Concentrations of trifloxystrobin in the diet were analysed twice during the treatment period for all doses. Mortality was checked twice per day and clinical signs were checked daily; body weight, food and water consumption were recorded weekly. Because this study was designed as a dose range-finding study, the extent of laboratory examinations do not meet the OECD 408/EPA (OPPTS) 870-3100 requirements for a 90-day study of oral toxicity in rodents. At the end of treatment, all animals were subjected to standard haematology and all surviving animals were subjected to a detailed necropsy, including collection of organs and tissues. Weights of the adrenals, kidneys, liver, ovaries, spleen, testes, and thymus were recorded. Although all organs were sampled, microscopic evaluations were limited to liver, spleen, and gross lesions. Food, water and the housing environment were controlled and monitored.

Trifloxystrobin was found to be homogeneously distributed and stable in the diet for at least 5 weeks at room temperature. One female at the lowest dose (500 ppm) died prematurely. Histopathological examination revealed no treatment-related cause of death (see below). There were no clinical observations that were related to the treatment. Depressed body-weight gain was recorded in males treated at 7000 ppm, resulting in a terminal weight which was 5.3% below control and an overall weight gain which was reduced by 20% (not statistically significant). The depression became particularly obvious towards the end of treatment. Body weights were not affected in other groups. Food consumption was slightly increased compared with that of controls for male mice at 500, 2000 and 7000 ppm. The increase in food consumption was evident by week 6, but increases became significantly different at weeks 9, 11 and 12 at all doses, and at week 13 at 500 ppm. Mean increases compared with values for controls for weeks 9–13 ranged from 6.5% to 23.8% at 500 ppm, 8.3% to 28.2% at 2000 ppm, and 26.4% to 46.0% at 7000 ppm. A slight increase was also observed in females at the highest dose (mean, 17.9%; range, 3.4–39.4% over weeks 1–13). The sponsor states that exact determinations were biased by food spillage in several groups. Food consumption ratios were not calculated for the group at 7000 ppm because of the food spillage, but no deviations were recorded for animals of both sexes for the groups at 500 and 2000 ppm compared with values for controls. Overall mean water consumption (weeks 1–13) was markedly increased (50%) in females at the highest dose, but water consumption in males was not affected.

No treatment-related effects on the haematological profile were found. Mean carcass weight for the males at 7000 ppm was slightly decreased (by 8%) compared with values for controls. Mean absolute weights of the liver were increased in males at 2000 ppm (23%) and 7000 ppm (30%), and in females at 2000 ppm (39%) and 7000 ppm (51%) (Table 12). Mean relative weights of the liver were increased in males at 2000 ppm (15%) and 7000 ppm (40%), and in females at 2000 ppm (30%) and 7000 ppm (49%). Elevated absolute weights of the spleen were found in males at 2000 ppm (11%) and 7000 ppm (8.5%), and in females at 2000 ppm (15.8%) and 7000 ppm (36.8%). Increased relative weights of the spleen were found in males at 7000 ppm (17.5%), and in females at 500 ppm (7.2%),

2000 ppm (8.2%) and 7000 ppm (35.3%) (Table 12). However, the increased relative weight of the spleen in females at 500 ppm was not correlated with histopathology and was not considered to be toxicologically relevant by the consulting pathologist.

An increased incidence of enlarged liver (two out of ten livers examined) and spleen (six out of ten spleens examined) was found in females at 7000 ppm. In the female (in the group at 500 ppm)that died on day 92 of the study, a mass in the small intestine, scarring of the liver and an enlarged spleen were observed. However, the consulting pathologist stated that the cause of death was probably invagination of a length of intestine into an adjacent portion producing obstruction of the bowel. The lesions of the liver in this animal were likely to be caused by enterotoxins associated with the ileus (obstruction of the intestines), and the extramedullary haematopoiesis of the spleen was considered to be concomitant to the intestinal changes, according to the consulting pathologist.

Microscopic findings in the 10 livers examined from each sex per group included hypertrophy of hepatocytes, predominantly of the centrilobular region, in males (minimal, one; moderate, two; marked, four) and females (minimal, three; moderate, seven) at 7000 ppm, and necrosis of single hepatocytes or small groups of hepatocytes in males at 2000 ppm (minimal, three; moderate, three) and 7000 ppm (minimal, two; moderate one) and females at 2000 ppm (minimal, two) and 7000 ppm (minimal, one; moderate, three). An increased incidence of haemosiderosis in the spleen was noted in males (minimal, five) and females (minimal, seven; moderate, one) treated at 7000 ppm. Extramedullary haematopoiesis was found at an increased incidence in the spleen of males at 2000 ppm (minimal, two) and in males (minimal, six; moderate, one) and females (six minimal, three moderate) at 7000 ppm. Other tissues were not examined. However, no other treatment-related microscopic findings were noted in all examined tissues from the 18-month study of carcinogenicity at a dietary concentration of 500 ppm or below.

The no-observed-adverse-effect level (NOAEL) in this dose range-finding study in mice was 500 ppm, equal to 77 and 110 mg/kg bw per day in males and females, respectively, on the basis of increased absolute and relative weights of the liver, and increased incidences of liver necrosis in both sexes, in addition to increased incidence of extramedullary haematopoiesis in the spleen of males (Gerspach, 1994a).

Table 12. Organ weights in mice given diets containing trifloxystrobin for 3 months

Organ	Dietary concentration (ppm)							
	0		500		2000		7000	
	Males	Females	Males	Females	Males	Females	Males	Females
Liver								
Absolute weight (g)	2.40	1.82	2.54	2.00	2.96[b]	2.53[a]	3.11[a]	2.75[a,b]
Relative weight (%)	5.90	5.90	6.00	6.20	6.80[a,b]	7.70[a,b]	8.30[a,b]	8.80[a,b]
Spleen								
Absolute weight (g)	0.082	0.095	0.082	0.105	0.091	0.110	0.089	0.130[b]
Relative weight (%)	0.201	0.308	0.195	0.330	0.209	0.333	0.236	0.416[b]

From Gerspach (1994a)

[a] $p < 0.01$, Lepage two-sample test

[b] $p < 0.01$, Jonckheere trend test

Rats

In a study conducted in compliance with the principles of GLP (with QA certification), groups of five male and five female Sprague-Dawley derived rats (Tif: RAIf (SPF) hybrids) were given diets containing trifloxystrobin (purity, 96.2%) at a concentration of 0, 200, 1000, 4000 or 12 000 ppm (equal to 0, 16.5, 84.4, 337 and 1074 mg/kg bw per day in males and 0, 16.4, 84.1, 327 and 1005 mg/kg bw per day in females, respectively) for 28 days. All animals were checked daily for mortality, health, and behaviour. Body weight, and food and water consumption were recorded weekly. Laboratory investigations (haematology, blood chemistry and urine analysis) were carried out on all surviving animals at each dose at the end of the treatment period. At scheduled sacrifice, all animals were subjected to macroscopic examination, including collection of organs and tissues. Organ weights were recorded for adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, and thymus, but because this was a dose range-finding study, no microscopic evaluations were performed. Food, water and the housing environment were controlled and monitored.

There were no deaths. During the treatment period, soft faeces were observed for all animals at 4000 and 12 000 ppm. The finding was reversible within the treatment period for females at 4000 ppm. Diarrhoea was observed in one male at 4000 ppm and in all males and one female at 12 000 ppm. Body-weight gain over the entire 4-week period was decreased in male rats at 1000 (13%), 4000 (22%) and 12 000 ppm (34%) as well as in females at 12 000 ppm (27%). There was a slight reduction in mean food consumption in weeks 1–4 of 4–6% below the respective control values for males at 1000 and 4000 ppm and for both sexes at 12 000 ppm. The mean food consumption ratios of animals at 12 000 ppm were lower than those of the control group at week 1 of treatment. Water consumption was not affected. There were no treatment-related effects on haematological parameters. Blood chemistry results, which were statistically significantly different from control values and were considered to be treatment-related, included: increased blood concentrations of glucose in males at 4000 ppm (17.8%) and 12 000 ppm (13.6%), and in females at 12 000 ppm (34.9%); increased serum concentrations of albumin in males at 4000 ppm (4.8%), and in animals of both sexes at 12 000 ppm (males, 7.8%; females, 5.9%); increased serum concentrations of cholesterol in animals of both sexes at 4000 ppm (males, 29.0%; females, 32.9%) and 12 000 ppm (males, 69.1%; females, 31.3%); increased serum concentrations of urea in females at 4000 ppm (22.9%) and 12 000 ppm (55.7%). All other minor fluctuations in blood chemistry were incidental and not related to treatment. There were no treatment-related effects on urine analysis parameters. Compared with the control group, mean carcass weights were 12% and 20% lower in males at 4000 and 12 000 ppm respectively, and 15% lower in females at 12 000 ppm. Mean relative weights of the liver were 13% and 31% higher in males at 4000 and 12 000 ppm and 15% higher in females at 12 000 ppm. Mean relative weights of the kidney were increased in males (15%) and females (9%) at 12 000 ppm. Mean relative weight of the adrenals was 20% higher in males at 12 000 ppm than in the controls. There were no treatment-related necropsy findings.

The NOAEL was 1000 ppm, equal to 84 mg/kg bw per day, on the basis of decreased body weight and body-weight gain and increased relative weight of the liver in males, and clinical signs and changes in clinical chemistry parameters in both sexes (Gerspach, 1994b). The study was considered to be supplementary, as it is a range-finding study and no histopathological examination was performed.

In a 28-day study of dermal toxicity, conducted in compliance with the principles of GLP with QA certification, groups of five male and five female Sprague-Dawley-derived rats (Tif:RAIf(SPF) were given trifloxystrobin (purity, 96.4%) administered dermally on 5 days per week for 4 weeks. Fur was clipped from the dorsal area of the rats' trunks over an area of at least 10% of the body surface on the day before the first application and weekly thereafter. Trifloxystrobin was applied as a suspension in 0.5% (w/v) carboxymethylcellulose in 0.1% (w/v) aqueous polysorbate 80 at a dose of 0, 10, 100 or 1000 mg/kg bw to the right side of the clipped area, and the vehicle only was applied to the left side of the clipped area, evenly dispersed on gauze patches, loosely covered with aluminium wrap and fastened to the body with adhesive tape. Dressings were removed after 6 h and the application areas were cleaned with lukewarm water. Food, water, and the housing environment were controlled and monitored. Animals were checked daily for clinical signs and mortality. Skin reactions were assessed at the application site approximately 17 h after removal of the gauze patches. Body weight and food consumption were recorded once weekly (on study days −7, 1, 8, 15, 22 and 28). Blood chemistry and haematological investigations were carried out on all surviving animals at each dose at the end of the treatment period. All control and treated animals were subjected to a detailed necropsy at the end of treatment, including collection of organs and tissues. Organ weights were recorded for adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, and thymus. Although all organs were sampled, microscopic evaluations were limited to kidneys, liver, pancreas, skin (both treated and untreated), spleen, thymus, and thyroid/parathyroid.

No treatment-related clinical signs or changes to behaviour were noted. One female animal at 10 mg/kg bw presented with transient crust and slight erythema, and two females at 1000 mg/kg bw presented with blisters on the skin application site. Since two females in the control group also had a similar skin reaction, these signs were likely to be caused by physical irritation. No other signs of local irritation were observed during the treatment period. There were no deaths during the study. No treatment-related changes were observed for mean body weights, food consumption and food consumption ratios. In females, there was a dose-related tendency to higher neutrophil and monocyte counts compared with values for controls and this was significant at 1000 mg/kg bw (neutrophils, 68.2%; monocytes, 73.0%). However, these findings were probably not toxicologically relevant given the absence of any histopathological abnormalities, and were likely to be incidental in nature. No other haematological parameters were affected by treatment with trifloxystrobin. There were minor changes in some of the blood chemistry parameters which were not dose-dependent and were within the normal range for historical controls. In males at 1000 mg/kg bw, the mean absolute and relative weights of the liver were increased by 17% and 15%, respectively, compared with controls. Mean absolute and relative weights of the kidney were also increased at 1000 mg/kg bw by 17% and 15%, respectively, compared with controls. No treatment-related macroscopic findings were noted. A lobular necrosis caused by incidental torsion of a hepatic lobe was observed but, according to the consulting pathologist, this lesion occurred spontaneously in the colony of rats used. No remarkable treatment-related microscopic changes were revealed. A low incidence of very slight local reactive lesions resulting from mechanical irritation caused by clipping of the fur was observed in both control and treated application skin sites.

In conclusion, repeated dermal treatment with trifloxystrobin did not result in irritation or any dermal toxicity. The NOAEL for systemic effects in male rats was 100 mg/kg bw per day on the basis of increased absolute and relative weights of the kidney and liver as supported by evidence of toxicity in the kidney and liver after oral administration of

trifloxystrobin. The NOAEL in female rats was 1000 mg/kg bw per day, since dermal application of trifloxystrobin at doses of up to and including 1000 mg/kg bw per day (a limit dose) resulted in no treatment-related toxicity (Gerspach, 1996).

In a 90-day study conducted in compliance with the principles of GLP and with quality assurance (QA) certification, groups of 15 or 25 Sprague-Dawley derived rats (Tif:RAlf, hybrids) of each sex were given diets into which trifloxystrobin (purity, 96.2%) had been homogeneously incorporated at a concentration of 0, 100, 500 or 2000 ppm (equal to 6.4, 30.6, and 127 mg/kg bw per day) in males and 0, 100, 500, 2000 or 8000 ppm (equal to 6.8, 32.8, 133 and 618 mg/kg bw per day) in females. The control group and the group receiving the highest dose (females, 8000 ppm; and males, 2000 ppm) included an additional 10 rats of each sex (i.e. total group size was 25 of each sex) that were kept on a control diet for a 4-week recovery period after 13 weeks of treatment. All other groups contained 15 animals of each sex. Trifloxystrobin concentrations in the diet were analysed twice during the treatment period for all doses. Mortality was checked twice per day and clinical signs daily; body weight, food, and water consumption were recorded weekly. Before the test, towards the end of the treatment period, and after recovery, all animals from the control group and from the group receiving trifloxystrobin at 8000 ppm were subjected to ophthalmology examinations (appearance of eye and periocular region, pupillary reflex). Observations and neurological examinations including functional observational battery (FOB) and motor activity were performed on 10 animals of each sex per group (15 animals in the control group and in the group at 8000 ppm) at weeks 4, 9, 13 and 17 (recovery animals only). The neurological examinations covered the functional domains of centra nervous sytem (CNS) activity, CNS excitation, sensorimotor functions (approach, touch, vision, audition, pain, vestibular), autonomic functions (pupillary reflex, body temperature), sensorimotor coordination (grip strength, landing foot splay) and physiological functions. At the end of the treatment and recovery periods, animals were subjected to haematology, clinical chemistry and urine analyses. At necropsy, the weights of the adrenal glands, brain, heart, kidneys, liver, ovaries, spleen, testes, thymus and thyroid/parathyroid were recorded and gross findings were noted. Microscopic evaluations were conducted on a range of tissues from all animals, including the animals that died during the test period or that had to be sacrificed in a moribund condition. In addition, a range of organs and tissues were specifically prepared for neuropathological assessment (glutaraldehyde perfusion and fixation) that was limited to animals in the control group and at the highest dose (five rats of each sex per group). Food, water and the housing environment were controlled and monitored.

Trifloxystrobin was found to be homogeneously distributed and stable in the diet for at least 5 weeks at room temperature. Some animals in the groups at 8000 ppm, 2000 ppm and the control group were found dead or had to be sacrificed. Five females in the group at 8000 ppm were found dead or had to be sacrificed in a moribund condition between days 28 and 34. At 2000 ppm, one female was found dead at day 16 and one male had to be sacrificed on day 35. In the control group, one male had to be sacrificed in a moribund condition on day 69 and one female was found dead on day 43. All deaths in the groups receiving trifloxystrobin at 2000 and 8000 ppm were considered to be treatment-related. Transient piloerection and soft faeces at week 1 were noted in all females at 8000 ppm. Hunched posture or hypoactivity were observed with moribund animals. There were no differences in ophthalmology findings between animals in the control group and animals at the highest dose.

Body-weight loss was associated with being in a moribund condition. At the end of dosing, body weights were statistically significantly decreased in males at 2000 ppm (13%) and in females at 8000 ppm (20%). The mean terminal body-weight gain was reduced by 9% and 20% in males at 500 and 2000 ppm and by 17% and 40% in females at 2000 and 8000 ppm, respectively (Table 13). At the end of the recovery period, body weights were similar in the control group and in the group receiving the highest dose owing to achieving a greater body-weight gain (23% and 60% above that in males and females in the control groups, respectively) after the high-dose animals were switched to control diets. Effects on body weight were partly associated with reduced food consumption. During the treatment period, food intake in males at 500 and 2000 ppm and in females at 2000 and 8000 ppm was lowered by approximately 5–10%. The resulting food consumption ratio was increased in females at 8000 ppm except for week 1 (5.8% at week 2 to 13.8% at week 13). During recovery, food intake of animals at the highest dose was 25–53% higher than that of animals in the control group. Water consumption was slightly reduced in males at 2000 ppm during weeks 1–4 (10.3–23.7%). Thereafter, the water intake was similar to values for animals in the control group. Overall, the mean water consumption of females at 8000 ppm was 11% lower than that of the control group, while it was similar to that of the control group during the recovery period.

FOB testing revealed no indications for a potential neurological or behavioural effect of trifloxystrobin. No changes of toxicological relevance were observed in any of the parameters associated with motor activity. Macro- and microscopic examination of tissues of the central and peripheral nervous system did not reveal any treatment-related neuropathological changes. Trifloxystrobin was considered to be nonneurotoxic in rats treated continuously for 90 days. There were minimal increases in erythrocyte parameters, including erythrocyte counts (4%) and haemoglobin concentration (3%) and a tendency to eosinophilia among females at 8000 ppm. These minor alterations were reversible within the recovery period.

There were some changes in clinical chemistry parameters consistent with a marginal effect on liver and kidney functions in males at 2000 ppm and in females at 2000 and 8000 ppm. Compared with values for controls, males at 2000 ppm had slightly increased plasma concentrations of creatinine (13.8% at week 14 and 3.7% at week 18), increased total bilirubin (44.8% at week 18 only), and increased cholesterol (28% at week 14), while

Table 13. Mean body-weight gain and weights of liver and kidneys in rats given diets containing trifloxystrobin for 90 days

	Dietary concentration (ppm)						
	Males			Females			
	100	500	2000	100	500	2000	8000
Body-weight gain (% of control)	104	91	80	96	104	83	60
Organ weight (% of control):							
Liver							
Absolute	107	105	106	94	98	107	109
Relative	102	113*	122*	95	96	113	139*
Kidney							
Absolute	105	102	97	96	97	95	89
Relative	100	108	112*	96	95	100	114*

From Gerspach (1995)

small reductions were noted in plasma globulin (9.6%) and protein concentrations (4.4%). Females at 2000 and 8000 ppm also had decreased plasma concentrations of globulin (8.7 and 11.5% respectively) and protein concentrations (5.3% and 4.3% respectively). In addition, females treated at 8000 ppm had increased concentrations of glucose (13.1%), urea (17.8%), potassium (by 10.1%), serum glutamic pyruvic transaminase (SGPT) (11.7%), and alkaline phosphatase activity (47.4%). These changes were partly reversible during recovery. Also, the urine excreted by females at 8000 ppm was slightly acidic. The most notable clinical chemistry change was the increased alkaline phosphatase activity, which may point to an adverse change in the hepatobiliary function of females at 8000 ppm.

One male at 2000 ppm and three females at 8000 ppm were emaciated at termination of treatment. Macroscopic examination revealed a small thymus in three out of 13 female animals at 8000 ppm. The consulting pathologist considered that the observation of a small thymus in the male animal was not toxicologically relevant. At 8000 ppm, one out of eight females had a small thymus after recovery. The mean relative weights of the liver were increased in males at 500 ppm (13%) and 2000 ppm (22%), and in females at 2000 ppm (13%) and 8000 ppm (39%). Mean relative weights of the kidney were also above control values in males at 500 ppm (8%) and at 2000 ppm (12%) and in females at 8000 ppm (14.2%). Liver and kidney weight changes were partly reversible after recovery. Mean relative weights of the heart were also increased in females at 8000 ppm both at 14 weeks (26%) and 18 weeks (21%).

Histopathological examination of moribund or dead animals revealed minimal perilobular hepatocyte hypertrophy in the livers of four out of five females and minimal to moderate acute tubular lesion in the kidneys in five out of five females at 8000 ppm; minimal to moderate atrophy of the pancreas (exocrine and endocrine) in one out of one male and female animals each at 2000 ppm, and minimal to marked pancreatic atrophy in five out of five females at 8000 ppm (two out of five associated with oedema); minimal to marked atrophy of the spleen in one out of one and three out of five females at 2000 and 8000 ppm (although one out of eleven control males showed a minimal splenic atrophy as well); minimal to moderate hypocellularity of the bone marrow, associated with haemorrhage in one out of one male and female animals at 2000 ppm and in five out of five females at 8000 ppm; minimal to moderate atrophy of the lymphatic tissue in one out of one female and five out of five females at 2000 and 8000 ppm, respectively; minimal to moderate atrophy of the salivary gland in one out of one male and female animals each at 2000 ppm, and in five out of five females at 8000 ppm; minimal to moderate mucosal atrophy affecting the small intestine in one out of five female animals and the large intestine in three out of five females at 8000 ppm; moderate atrophy of the uterus in one out of one female at 2000 ppm and three out of five females at 8000 ppm; minimal to moderate atrophy of the ovary in one out of one and three out of five females at 2000 and 8000 ppm, respectively; moderate atrophy of the adenohypophysis in one out of one female at 2000 ppm and three out of five females at 8000 ppm; moderate to marked atrophy of the thymus in one out of one male and female animals each at 2000 ppm and moderate to marked thymic atrophy in five out of five females at 8000 ppm.

At scheduled sacrifice, there were a few histopathology findings among females at 8000 ppm and among both sexes at 2000 ppm, but no changes were seen at the lower dose. Among the changes observed were minimal hepatocellular hypertrophy in males dosed at 2000 ppm (five out of 10) and in females dosed at 8000 ppm (seven out of eight), minimal to moderate atrophy of the pancreas in two out of 10 males, in one out of nine females at

2000 ppm, and in seven of eight females at 8000 ppm. In addition, one female treated at 8000 ppm had minimal atrophy of the salivary gland. In the recovery group there were minimal to moderate atrophy of the endocrine pancreas in two out of 10 males at 2000 ppm and minimal atrophy of the thymus and the uterus in one out of eight females each at 8000 ppm. The liver pathology findings among males at 2000 ppm and females at 8000 ppm are consistent with some of the changes in clinical chemistry parameters and the increased relative weight of the liver.

The NOAEL was 500 ppm, corresponding to 31–33 mg/kg bw per day, on the basis of statistically significantly decreased body-weight gains, increased relative liver weights, changes in clinical chemistry, and liver histopathology findings in addition to pancreatic atrophy at the next higher dose of 2000 ppm. In males at 500 ppm, the decrease in body-weight gain was minor (<10%) and not statistically significant; also the slightly increased relative weight of the liver was not corroborated by liver histopathology or clinical chemistry findings (Gerspach, 1995).

Dogs

In a study conducted in compliance with the principles of GLP and with QA certification, groups of two male and two female beagle dogs were given gelatin capsules containing trifloxystrobin (purity, 96.2%) at a dose of 0, 20, 50 or 150 mg/kg bw per day orally once daily, 7 days per week for 28 days. At day 29, dogs in the control group and at 20 and 50 mg/kg bw per day were sacrificed. Owing to a lack of toxic effects, the dose for dogs in the group receiving trifloxystrobin at 150 mg/kg bw per day was increased to 500 mg/kg per day for an additional 21 days. Food, water and the housing environment were controlled and monitored. All animals were checked daily for behavioural signs and mortality. The body weight and food consumption of all animals was recorded at weekly weighing sessions. Food consumption ratios were calculated as: weekly food consumption (g)/body weight (kg) × 7 (g food/kg bw per day). Eye examinations were performed in all animals before the test and at week 4. Laboratory investigations (haematology, blood chemistry and urine analysis) were carried out on all surviving animals at each dose at the beginning of the study and at weeks 4 and 7. All animals were subjected to a detailed necropsy at the end of the test period and organ samples were taken for microscopic examination. Only the following organs were microscopically examined: brains, heart, liver, kidneys, testes, ovaries, spleen, thymus adrenal gland, thryoid, parathyroid gland, and any tissues with gross lesions.

There were no deaths during the study. During the treatment period, vomiting was observed in the males and females at 150/500 mg/kg bw per day on several occasions. There was also increased incidence and severity of diarrhoea noted at 500 mg/kg bw per day. Mean body weights were lower (by 3.1%) in male animals given trifloxystrobin at 150/500 mg/kg bw per day at week 4 compared with before the start of treatment. A slight body-weight loss was noted for one male at 50 mg/kg bw per day at the end of the treatment (2.2%), and for one male at 150/500 mg/kg bw per day (6.25%), and a transient loss was observed at weeks 1 and 2 (by 3.1%) for one male at 150/500 mg/kg bw per day, compared with before the test. Mean body-weight gains of treated females were similar to those of the controls. Compared with before the test, mean food consumption was reduced maximally at week 3 by 25% for one male at 50 mg/kg bw per day, and at week 1 by 25% for one female and by 55% at week 2 for one male at 150/500 mg/kg bw per day. Food intakes were partially returned to values recorded before the test by the end of treatment. Lower food

consumption ratios were noted for males at 50 mg/kg bw per day (week 1, by 4.8%; week 2, by 10.6%; week 3, by 14.1%; and week 4, by 5.3%) and 150/500 mg/kg bw per day (week 1, by 21.9%; week 2, by 24.0%; week 3, by 23.5%; week 4, by 15.5%) during the treatment period. There were no treatment-related findings in the conjunctivae, sclera, cornea, lens, and fundus and no alterations of the pupillary reflex.

Treatment with trifloxystrobin had no effect on the haematology, blood chemistry or urine analysis parameters investigated. Mean absolute and relative weights of the liver were slightly increased in animals of both sexes at 150/500 mg/kg bw per day (Table 14), while mean absolute and relative weights of the spleen were increased in females only at this dose. The last finding in the spleen was confirmed by histological changes consisting of moderate congestion of the splenic red pulp in three out of four animals in the at the highest dose. The sponsor considered the splenic congestion to be an agonal condition known to occur as a result of the method of euthanasia, and was not considered of toxicological relevance. However, the correlation between congestion and increased spleen weight in the females at the highest dose and the lack of similar findings at the other doses suggest that the histopathology changes are treatment related. There were no other microscopic treatment-related effects and no remarkable findings on necropsy.

The NOAEL was 50 mg/kg bw per day on the basis of clinical signs (diarrhoea and vomiting), decreased body-weight gain, and measures of liver and spleen weights in both sexes in addition to increased splenic congestion in females at the highest dose (Altmann, 1994).

In a study conducted in compliance with the principles of GLP with QA certification, four male and four female beagle dogs were fed gelatin capsules containing trifloxystrobin (purity, 96.2%) once per day at a dose of 5, 30, 150 and 500 mg/kg bw per day for 91 days. After experiencing significant vomiting and decreased food consumption during the first 10 days, dogs in the group receiving trifloxystrobin at 500 mg/kg bw per day were given two capsules per day (each equivalent to 250 mg/kg bw per day), one at 2 h after feeding and one at approximately 3 h thereafter. Owing to severe body-weight loss in the group at

Table 14. Organ weights and organ-to-body-weight ratios in dogs given capsules containing trifloxystrobin for 28 days

	Dose (mg/kg bw per day)			
	0 (control)	20	50	150/500
Males				
Liver:				
Organ weight (g)	323.3	321.2	317.7	344.4
Organ-to-body-weight ratio (%)	2.98	2.94	3.41	3.85*
Kidney:				
Organ weight (g)	53.80	50.62	46.38	49.89
Organ-to-body-weight ratio (%)	0.49	0.45	0.50	0.56*
Females				
Liver:	261.1	280.1	247.1	345.5
Organ weight (g)	2.92	3.34	3.34	3.91*
Organ to body-weight ratio (%)				
Spleen:				
Organ weight (g)	24.31	27.87	25.09	41.19
Organ to body-weight ratio (%)	0.27	0.34	0.34	0.47*

From Altmann (1994)

* Statistical significance using the Jonckheere test ($p < 0.05$)

500 mg/kg bw per day, treatment for certain animals had to be discontinued for a few days at around the middle of the treatment period. Concentrations in the capsule were adjusted to maintain appropriate dosages according to body weight-gain/-loss of the animals. Mortality was checked twice per day and clinical signs daily; body weights were recorded weekly; food consumption was determined daily and reported as weekly means. Before the test and towards the end of the treatment period, all animals were subjected to ophthalmology examinations. Before the test, and at week 7 and week 13, all animals were subjected to haematology, clinical chemistry and urine analyses. After 90 days of treatment, all control and surviving treated animals were subjected to a detailed necropsy, including collection and microscopic evaluations of organs and gross lesions. Organ weights were recorded for adrenals, brain, heart, kidneys, liver, ovaries, spleen, testes, thymus, and thyroid/parathyroid.

One male at 500 mg/kg bw per day dose had to be sacrificed on day 66 owing to reduced food consumption, body-weight loss, and reduced locomotor activity. Vomiting was increased in a dose-related manner in animals of both sexes in week 1 (150 mg/kg bw per day: 15 occurrences observed in males, 12 in females; 500 mg/kg bw per day: 28 occurrences observed in males, 23 in females), followed by moderate to severe diarrhoea throughout the whole treatment period. Traces of blood were observed twice in the faeces of one male at 500 mg/kg bw per day at week 13. There were no differences in ophthalmology findings between control and treated animals.

A dose-dependent loss in body weight was recorded for males and females at 150 and 500 mg/kg bw per day. Normal body-weight development returned for animals in the group receiving trifloxystrobin at 150 mg/kg bw per day after about 4 weeks of treatment in males and after 6 weeks in females, but pre-test values were not reached until the end of the treatment period. Compared with values obtained before the test, mean body-weight loss at study termination for males and females was 0.4 kg and 0.38 kg at 150 mg/kg bw per day and 3.37 kg and 2.21 kg at 500 mg/kg bw per day, respectively. Body-weight development was not affected in animals at 5 or 30 mg/kg bw per day.

Food consumption of animals of both sexes at 500 mg/kg bw per day was markedly reduced during the first 2 weeks. To avoid excessive weight loss, feeding periods (normally about 3 h) were partly prolonged and food consistency was changed to a 1 : 1 mixture of pellets and powder. In males, force-feeding and partial discontinuation of treatment was also necessary. Food consumption in animals of both sexes at 500 mg/kg bw per day was reduced by as much as 15.7–43.7% in males and 18.9–37.8% in females during weeks 1–13. Mean food consumption was also moderately reduced at 150 mg/kg bw per day in males (by 30% at week 1 returning to control levels by week 9) and females (by 23% at week 1 and 45% at week 2, returning to control levels by week 10). Food consumption in the groups receiving the two higher doses returned to normal levels during the second half of the treatment period. During the first 3 weeks, a slight and transient reduction (by less than 12%) was also noted in females at 30 mg/kg bw per day dose, mainly owing to one animal. As body-weight development was not affected and no other toxicological findings were noted in this group, the transiently reduced food consumption in females at 30 mg/kg bw per day was not considered to be an adverse effect.

A slight hypochromic anaemia, in the form of lowered erythrocyte count, haemoglobin and erythrocyte volume fraction values (each by about 20–23%), was noted in all males and in one female at 500 mg/kg bw per day. Slightly reduced numbers of eosinophils (by

65% and 40% at weeks 7 and 13, respectively) and increased platelet counts (by 67% at week 13) were noted in males at 500 mg/kg bw per day. One male at 500 mg/kg bw per day had a leukocytosis with markedly increased numbers of neutrophils and monocytes. On the basis of increases in platelets, decreases in basophils, and increases in monocytes, it is likely that some of the males given trifloxystrobin at 500 mg/kg bw per day had an acute infection, possibly resulting from the overall poor health status of this group.

In the males and females at 500 mg/kg bw per day, there were significant decreases in several serum clinical chemistry parameters including creatinine, bilirubin, protein, albumin, and cholesterol, in addition to increases in concentrations of triglycerides (Tables 15 and 16). At 150 mg/kg bw per day, concentrations of triglycerides were increased in males and females, while concentrations of creatinine and cholesterol were decreased in females.

Table 15. Selected clinical chemistry changes (mean and % of control) in male dogs given capsules containing trifloxystrobin for 90 days

	Dose (mg/kg bw per day)				
	0	5	30	150	500
Creatinine (µmol/l)	76.15	73.73	69.73	67.48*	40.28*
Change at week 7		97%	92%	89%	53%
Creatinine (µmol/l)	74.93	72.55	77.78	78.30	46.08*
Change at week 13		97%	104%	104%	61%
Total bilirubin (µmol/l)	2.38	2.02	1.73*	1.96	1.51*
Change at week 13		85%	73%	82%	63%
Protein (g/l)	59.0	59.60	57.90	59.70	48.30*
Change at week 13		101%	98%	101%	82%
Albumin (g/l)	33.19	33.11	33.30	31.49	27.18*
Change at week 13		100%	100%	95%	82%
Cholesterol (mmol/l)	4.12	3.40*	3.72	4.09	2.84*
Change at week 13		83%	90%	99%	69%
Triglycerides (mmol/l)	0.38	0.35	0.58*	0.68*	0.65*
Change at week 13		92%	153%	179%	171%

From Altmann (1996)
* Statistical significance using the Wilcoxon test ($p < 0.05$)

Table 16. Selected clinical chemistry changes (mean and % of control) in female dogs given capsules containing trifloxystrobin for 90 days

	Dose (mg/kg bw per day)				
	0	5	30	150	500
Creatinine (µmol/l)	81.13	87.20	80.88	67.03*	53.83*
Change at week 13		107%	100%	83%	66.5%
Total bilirubin (µmol/l)	2.85	2.73	2.25	2.14	1.90*
Change at week 7		96%	79%	75%	67%
Protein (g/l)	55.84	58.68	59.71	57.78	48.64*
Change at week 13		105%	107%	103%	87%
Albumin (g/l)	33.16	34.46	34.86	33.35	28.02*
Change at week 13		104%	105%	101%	84%
Cholesterol (mmol/l)	3.66	4.08	3.93	2.83*	2.49*
Change at week 13		111%	107%	77%	68%
Triglycerides (mmol/l)	0.36	0.46	0.40	0.64*	0.59
Change at week 13		128%	111%	178%	164%

From Altmann (1996)
* Statistical significance using the Wilcoxon test ($p < 0.05$)

The only notable change in the group at 30 mg/kg bw per day was a slight increase in concentrations of triglycerides among males; however, this increase is considered to be incidental since pre-test levels were also high in this group. There were other changes (not shown here) including small decreases (generally <20%) in plasma concentrations of calcium, potassium, and phospholipids. Collectively, these changes may reflect a state of perturbed metabolism secondary to gastrointestinal problems, dehydration, lack of nutrition, and partial starvation. There were also isolated incidences of statistically significantly decreases in activities of serum liver enzymes that are not considered to be toxicologically relevant because a decrease was observed rather than the anticipated increase associated with liver toxicity; also, the decreases may be incidental, owing to unusually high levels in the control animals. In females at 500 mg/kg bw per day, there was a slight decrease in urine pH. Urine analysis profiles in other treatment groups were not altered.

Several organ weights and organ: body-weight ratios were affected. Compared with controls, carcass weights were moderately reduced in the group receiving trifloxystrobin at 500 mg/kg bw per day (males, by 35.7%; females, by 23.3%) and slightly lowered (by 5%) in females at 150 mg/kg bw per day. Mean relative weights of the liver increased dose-dependently at 150 mg/kg bw per day (males, by 33.6%; females, by 18.3%) and 500 mg/kg bw per day (males, by 60.9%; females, by 40.3%). Absolute weights of the liver were also increased (32.6%) in males at 150 mg/kg bw per day. The following changes were also seen in males and females at 500 mg/kg bw per day: increased relative weights of the kidney, by 36% and 21%; decreased absolute weights of the heart, by 50% and 31%; and decreased absolute (by 73% and 44%) and relative (by 61% and 30%) thymus weights, respectively. In addition, males at the same dose had increased relative weights of the adrenals (by 77%) and decreased absolute (by 68%) and relative (by 45%) testis weights. The consulting pathologist considered that the weight/relative weight changes in heart, kidneys, and adrenals were incidental, not dose-related, and not associated with histopathology findings. Therefore, the changes were considered to reflect the poor nutritional status and emaciation of the respective animals rather than a toxic effect; it should be noted, however, that relative weights of the kidney were also increased in the 3-month study in rats.

At necropsy, three out of four males and two out of four females at 500 mg/kg bw per day presented with emaciation of the body. Hair loss was also noted in one female in the same group. The male dog which was prematurely sacrificed in a moribund state had an enlarged gall bladder, which correlated with a moderate hyperplasia of the epithelium. Additional findings in this animal included enlargement of the adrenal glands, mottled stomach, and dilatation of the large intestines. There were no other treatment-related gross pathology findings.

There were several microscopic pathology findings that seemed to reflect both exposure to trifloxystrobin and effects of emaciation in the group at 500 mg/kg bw per day. The following observations probably resulted from emaciation: myopathy of skeletal muscle and atrophy of the cervical, mesenteric, and popliteal lymph nodes. Minimal hypertrophy of hepatocytes was observed in three out of four males at 150 and 500 mg/kg bw per day and in all females at 500 mg/kg bw per day. Minimal to moderate hyperplasia of the epithelium of the gall bladder was also noted in two out of four males and three out of four females at 500 mg/kg bw per day. In addition, a marked cytoplasmic vacuolization of hepatocytes, minimal erosion of the small intestine mucosa, moderate inflammatory cell infiltration and a moderate and focal dilatation of the intestinal glands was seen in the prematurely sacrificed male at 500 mg/kg bw per day. Furthermore, a minimal hypocellularity of the bone

marrow and a moderate atrophy of the white pulp of the spleen were detected in this animal. Atrophy was considered by the consulting pathologist to be secondary to the reduced food intake and the resulting emaciation in the animals at 500 mg/kg bw per day causing increased incidences of the following: minimal atrophy in the mesenteric lymph nodes of one out of four females and minimal to moderate atrophy in the thymus of three out of four females; atrophy of the skeletal muscle, reported as myopathy, in three out of four males (minimal to moderate) and in two out of four females (minimal); and moderate prostatic atrophy and moderate tubular atrophy of the testes (associated with a secondary moderate or marked reduction of spermatozoa in the lumen of epididymides) in all male dogs. All other changes were considered by the consulting pathologist to be incidental and/or occurring commonly in the colony of beagle dogs, and were not treatment-related.

The NOAEL was 30 mg/kg bw per day in both sexes on the basis of clinical signs of vomiting, body-weight loss, and increased absolute and relative weights of the liver, accompanied by hepatocyte hypertrophy (Altmann, 1996).

In a study conducted in compliance with the principles of GLP with QA certification, groups of beagle dogs were given gelatin capsules containing trifloxystrobin (purity, 96.4%) at a dose of 0, 2, 5, 50 or 200 mg/kg bw per day orally, once daily, 7 days per week for 52 weeks. The dogs received the capsules approximately 1–2 h after feeding. Capsules were prepared in about weekly intervals adjusted to body weights of the preceding week. Urine was collected by catheterization. Checks for mortality were made twice per day and for clinical signs daily; body weights were recorded weekly; food consumption was determined daily and reported as weekly means. At pre-test and towards the end of the treatment period, all animals were subjected to ophthalmology examinations (external inspection, examination of lens, iris and fundus with ophthalmoscope, pupillary reflex, examination of third eye lid after local anaesthesia). At pre-test, week 13, 26 and 52, all animals were investigated for haematology, clinical chemistry and urine analyses. Animals were fasted 16 h before blood collections. At scheduled sacrifices, the surviving control and treated animals were subjected to detailed necropsy and histopathology. Organ weights were recorded for adrenal glands, brain, heart, kidneys, liver, ovaries/testes, spleen, thymus, and thyroid/parathyroid.

Trifloxystrobin was found to be stable under the conditions of the test. There were no deaths during the study. Diarrhoea was increased in frequency and severity among animals of both sexes at 200 mg/kg bw per day and to a lesser degree in males at 50 mg/kg bw per day (Table 17). Slightly loose stools were also noted in females at 50 mg/kg bw per day. Vomiting occurred in animals of both sexes at 200 mg/kg bw per day, but more severely in females. Brownish discolouration of hair and skin of the paws, thorax and abdomen was noted in all animals at 200 mg/kg bw per day and in six out of eight animals at 50 mg/kg bw per day (Table 17). Discolouration was observed first on the paws of animals at the highest dose at week 15 and was seen throughout the entire observation period. Towards the end of the study the intensity of staining decreased and discolouration was mainly restricted to the paws. In the group at 50 mg/kg bw per day, the finding was generally restricted to paws and occurred in a transient manner or at the end of the study. The stain could not be removed by washing. There were no treatment-related changes in ophthalmology findings.

In females at 50 and 200 mg/kg bw per day, mean overall body-weight gain was slightly depressed, with the reduction being statistically significant (from weeks 2–7 inclusive) and was more pronounced earlier in the study. For instance, in females at 50 and 200 mg/kg bw per day, body-weight gain was reduced by 35.3% and 97.5% at week 2, by

Table 17. Incidences of clinical signs in dogs given capsules containing trifloxystrobin for 12 months

Clinical sign	Dose (mg/kg bw per day)									
	0		2		5		50		200	
	Males	Females	Males	Females	Males	Females	Males	Females	Males	Females
Diarrhoea[a]										
Moderate	<5	<10	0	0	0	0	>70	<20	>350	>350
Severe	0	0	0	0	0	0	<20	<5	>180	>80
Vomiting[a]	0	0	0	0	0	0	0	4	18	113
Dark discolouration[b] of coat of:										
Paws	0	0	0	0	0	0	3	3	4	4
Thorax	0	0	0	0	0	0	0	2	4	4
Abdomen	0	0	0	0	0	0	0	1	4	4
Dark discolouration[b] of skin of above regions	0	0	0	0	0	0	1	3	4	4

From Altmann (1997)

[a] Number of occurrences

[b] Number of animals affected within each group (n = 4)

18.5% and 26.1% at week 26, and by 22.8% and 19.9 at week 52, respectively. Body-weight gain in males was unimpaired by treatment.

Mean food consumption was slightly and transiently reduced in males and moderately reduced in females at 200 mg/kg bw per day with the largest decrease being seen at the beginning of the study. For instance, during week 2, food intake in males and females at 200 mg/kg bw per day was below values for controls by 10.3% and 27.3%, respectively, and remained slightly depressed until the end of treatment in females (by 6.9% at week 52). A slightly reduced food consumption was also noted in females at 50 mg/kg bw per day (by 10.3% at week 52). Food consumption ratios fluctuated in both sexes at 200 mg/kg bw per day and in females at 50 mg/kg bw per day, reflecting the reduced food intake observed in these animals.

Lower eosinophil counts were recorded for males at 200 mg/kg bw per day from week 13 onwards, with a statistically significant difference compared with those of controls at week 52 (by 42.6%). Compared with controls, females at 200 mg/kg bw per day had statistically significantly higher platelet counts at weeks 26 (by 6.5%) and 52 (by 9.0%). However, these platelet counts did not differ appreciably from those recorde before the start of the test and were therefore judged by the sponsor to be unrelated to treatment. Other differences between the means that attained a level of statistical significance were not likely to be related to treatment as the magnitude of the changes was too small to be toxicologically relevant and/or the changes occurred without any relation to the dose administered or to the duration of treatment.

Some of the clinical chemistry parameters were changed in males at 50 mg/kg bw per day and in males and females at 200 mg/kg bw per day; the changes in males were consistent throughout the study. Compared with controls, males at 50 mg/kg bw per day had lower plasma concentrations of albumin (by 3–9%) and higher activities of alkaline phosphatase (by 13%, 29% and 53% at weeks 13, 26 and 52, respectively). Compared with controls, males at 200 mg/kg bw per day also had a lower plasma concentrations of albumin (by 7–13%), higher activities of alkaline phosphatase activity (by 28%, 72%, and 117.0%), and higher plasma concentrations of of triglycerides (by 46%, 51% and 97%) throughout the study (at weeks 13, 26 and 52, respectively); in addition, there was a slight increase in

concentrations of chloride at week 52 (by 4.8%). Some treatment-related changes were also seen among females throughout the study, but were noted only at 200 mg/kg bw per day and were limited to a higher plasma concentrations of triglycerides (by 70%, 66% and 43% at weeks 13, 26 and 52, respectively) and a higher alkaline phosphatase activity (by 26%, 70%, and 82% at weeks 13, 26 and 52 respectively). Other sporadic statistically significant differences were not dose-related, were observed only at a single time-point, and/or the magnitude of the changes were small. No treatment-related effects were found in any of the qualitative or quantitative urine analysis parameters.

No treatment-related gross lesions were found. The findings of "lung nodule" and "lung mottled" were not associated with microscopic findings and therefore were not considered treatment-related by the consulting pathologist.

Mean absolute and relative weights of the liver were increased by nearly 15–39% in males and females at 50 or 200 mg/kg bw per day (Table 18). The effects on liver weights correlated with some of the clinical chemistry and histopathology findings.

At 50 and 200 mg/kg bw per day, there was a slight increase in mean absolute (by 21.8% and 25.4%) and relative (by 18.7% and 31.6%) testes weights. However, the testes weights in the treated groups were within the range for historical controls, while the testes weights in the control group were at the lower end of the range for historical controls. Additionally, there were no pathology findings in the testes and the increased weight was therefore not likely to be treatment-related. In the absence of histological changes and because absolute weights were similar to those of the controls, the significant increase in relative weight of the adrenals (by 24.4%) in males at 200 mg/kg bw per day was probably incidental in nature and unrelated to treatment. Also, the increased weight of the spleen (by 58.15%) in females at 2 mg/kg bw per day was not dose-dependent and not likely to be treatment-related.

An increase in the incidence and/or severity of hepatocellular hypertrophy was found in the livers of animals of both sexes at 200 mg/kg bw per day and in females at 50 mg/kg bw per day (Table 19). Minimal to slight bone marrow hypocellularity occurred at a higher incidence in males and females in the group receiving trifloxystrobin at a dose of 200 mg/kg bw per day (Table 19) with no attendant effect on erythrocyte or leukocyte populations.

A dose-related tan discolouration of the skin was found in animals at 50 and 200 mg/kg bw per day. The author of the study considered it to be a physicochemical "dyeing" effect of the test article. The discolouration of the body surface areas appeared to be resistant to formalin solution, but disappeared after normal histopathological process-

Table 18. Absolute and relative weights of the liver in dogs given capsules containing trifloxystrobin for 12 months

Dose (mg/kg bw)	Males		Females	
	Absolute weight (g)	Relative weight (%)	Absolute weight (g)	Relative weight (%)
0 (control)	317.4	2.81	282.2	2.78
50	375.1	3.22	341.5	3.48
200	421.9*	3.90*	363.3	3.79*

From Altmann (1997)
* $p < 0.05$; Jonckheere's test

Table 19. Incidences of microscopic findings in dogs given capsules containing trifloxystrobin for 12 months

Finding	Males					Females				
	0	2	5	50	200	0	2	5	50	200
Tissues examined	4	4	4	4	4	4	4	4	4	4
Treatment-related incidences										
Liver: hepatocellular hypertrophy	1	1	1	1	3	0	0	0	3	4
Bone marrow: hypocellularity	1	1	1	1	3	2	1	1	2	4

From Altmann (1997)

ing. On microscopic examination, no discolouration and no treatment-related histopathological changes were seen in tissue sections stained in a standard manner with haematoxylin and eosin. In the absence of any pathological consequences, this finding was considered to be of no toxicological relevance.

Cross-sections of testes as well as the seminiferous tubules appeared slightly larger in the groups receiving trifloxystrobin at 50 and 200 mg/kg bw per day than in the control group. However, histological appearance, maturity, cyclic development and spermatogenic stages were normal in all animals. No pathological changes were found in any of the examined testes. The consulting pathologist concluded that the difference in testis size reflected normal physiological variations and was of no toxicological relevance.

The NOAEL was 5 mg/kg bw per day in both sexes based on increased absolute and relative weights of the liver (in both sexes), hepatocellular hypertorphy (in females), biochemical changes (in males), diarrhoea (in both sexes), and reduced body-weight gain (in females) at the next higher dose (Altmann, 1997).

2.3 Long-term studies of toxicity and carcinogenicity

Mice

In a study conducted in compliance with the principles of GLP with QA certification, groups of 70 male and 70 female mice (Tif:MAGf, hybrids) were continuously fed diets containing trifloxystrobin (purity, 96.2%) at a dose of 0, 30, 300, 1000 and 2000 ppm (equal to 3.9, 39.4, 131.1 and 274 mg/kg bw per day in males and 3.5, 35.7, 124.1 and 246 mg/kg bw per day in females, respectively) for 18 months. For the carcinogenicity evaluation, 50 mice of each sex per dose were maintained for 18 months. The remaining animals were subjected to haematological evaluations at weeks 53 (10 of each sex per group) and 79 (10 of each sex per group). In addition, blood smears were prepared from all surviving animals that were involved in the carcinogenicity evaluation at terminal sacrifice. Concentrations of trifloxystrobin in the diet were analysed periodically throughout the study. Samples of the diet prepared for the first 4 weeks of the study were analysed for stability (for 5 weeks) and homogeneity of trifloxystrobin. Mortality was checked twice per day (once at weekends and holidays) and clinical signs daily; body weight and food consumption were recorded weekly for the first 3 months and monthly thereafter; water consumption was recorded monthly. At scheduled sacrifices the surviving control and treated animals were subjected to detailed necropsy and histopathology. Organs weights were recorded for adrenal glands, brain, kidneys, liver, ovaries/testes, and spleen.

Table 20. Survival to termination necropsy in mice given diets containing trifloxystrobin for 18 months

Dietary concentration (ppm)	Males		Females	
	No.	%	No.	%
0 (control)	43	86	42	84
30	45	90	36	72
300	40	80	45	90
1000	45	90	45	90
2000	46	92	42	84

From Gerspach (1997a)

Table 21. Summary of cumulative body-weight gain (% of control) in mice fed diets containing trifloxystrobin for 18 months

Week	Dietary concentration (ppm)							
	Males				Females			
	30	300	1000	2000	30	300	1000	2000
Week 13	100	101	96	90**	96	92	87**	86**
Week 40	98	90	92	93	89	81	83**	85**
Week 78	95	95	92	91	92	86	98	89

From Gerspach (1997a)
** Statistically significant ($p < 0.01$)

Trifloxystrobin was found to be homogeneously distributed and stable in the diet for at least 5 weeks at room temperature. Concentrations in the diet ranged from 82% to 116% of the nominal values.

The survival rate was not affected by treatment (Table 20). The relatively higher number of deaths occurring in females at 30 ppm was considered incidental. No treatment-related clinical signs or behavioural changes were observed at any dose.

Relative to control, body-weight gain was slightly but consistently reduced (by 5–10%) in males at 2000 ppm (Table 21). The effect became obvious particularly during the growth phase, i.e. the first 9 months, with statistical significance being achieved only at weeks 7, 10 and 13. After 3 months of treatment, the mean body-weight gains were decreased in females by 10% at 1000 ppm and by 14% at 2000 ppm when compared with controls. The difference between the group at 2000 ppm and the control group exceeded 20% on several occasions. Body-weight development of females at 300 ppm was also affected, but to a lesser extent in magnitude and significance. Decreased body-weight gain in this group started around week 10 and persisted throught the study at a magnitude of nearly 10–20%; however, statistical significance was only achieved at weeks 10, 43, 47 and 67 (by 21.4%, 20.7%, 18.1% and 14.2%). There were no other toxicological findings in the same group (females at 300 ppm). The decreased body-weight gain of females at 300 ppm is most likely to be treatment-related but, on its own, this effect is not sufficiently robust to be considered as adverse. In the groups of males at 30, 300 and 1000 ppm and the group of females at 30 ppm, body-weight development was similar to that in the control group. Minor variations in these groups were incidental.

Cumulative food consumption in females at 2000 ppm throughout the study was 7% below that of the control group. No treatment-related effects were observed at any other

dose. The occasional occurrence of statistically significant differences was influenced by food spillage in the respective groups. Water consumption was not affected by treatment.

At both weeks 53 and 79, no treatment-related effects on haematology parameters were observed at any dose. The trends to lower mean cell haemoglobin concentration (MCHC) values in males at 2000 ppm at weeks 53 (by 2.7% compared with controls) and 79 (by 2.95% compared with controls) and at 1000 ppm at week 79 (by 3.6% compared with controls) in addition to lower haemoglobin concentrations (by 7%compared with controls) at week 53 in females at 2000 ppm were of no toxicological relevance owing to the absence of dose-dependency or the low magnitude of the effect. Lymphatic leukaemia, which was observed with an incidence of one out to three animals per group in all groups (including the control group), is known to occur spontaneously at a low incidence in the colony of mice, according to the sponsor.

Compared with the controls, carcass weight was reduced in females at 2000 ppm by 13% at interim sacrifice (week 39). Probably as a result of this, relative weights of the liver and spleen were increased by 14% and 34%, respectively. Absolute and relative weights of the liver were significantly increased in males at 2000 ppm (by 33% and 27%) and to a lesser extent also at 1000 ppm (by 15% and 13%). At terminal sacrifice (week 79), absolute and relative weights of the liver showed significantly increased values in males at 2000 ppm (by 22% and 25%) and in females (by 7% and 12%). A similar effect was also observed in females at 1000 ppm (by 7% and 10%), but only minimally (by 4–5%) in the respective group of males. There was a minimal trend to increased relative weights of the kidney in females at 2000 ppm at week 79 (11.4%), but this is not likely to be of toxicological relevance owing to the absence of any other relevant findings in this organ.

A slightly increased number of males and females with enlarged liver was present in the group receiving trifloxystrobin at 2000 ppm, which corresponded with hepatocellular hypertrophy in some animals. There were no other treatment-related gross macroscopic findings. The slight decrease in masses and/or nodules of the liver in animals of both sexes at 1000 and 2000 ppm was likely to be incidental. These changes corresponded microscopically to various neoplasias of the liver.

Microscopic evaluation revealed treatment-related findings in liver only (Table 22). The incidence of hepatocellular hypertrophy was significantly increased in males and females at 2000 ppm. The severity of this change, however, was similar in control and treated animals. Incidences of single-cell necrosis (including minute clusters of necrotic hepatocytes) were significantly increased in males at 1000 ppm and in males and females at 2000 ppm. The incidence of focal liver necrosis was slightly increased in females at 2000 mg/kg. The severity of fatty change was increased in males at 2000 ppm. Most of these effects had already been detected at interim sacrifice (9 months). In treated female mice, fatty change and inflammatory cell infiltration of the liver were decreased in incidence but occurred in numerous animals.

Other observations not considered to be toxicologically relevant by the consulting pathologist included: an increased incidence of tubular casts in kidneys in females at 2000 ppm (83.3%) might be indicative of a mild stage of chronic progressive nephropathy, if it were associated with tubular atrophy. However, the incidence of tubular atrophy was not increased in this group. Chronic reactive hyperplasia was increased in mesenteric lymph nodes of females at 1000 and 2000 ppm. At both doses, 17 out of 47 mesenteric lymph

Table 22. Microscopic findings in the livers of mice given diets containing trifloxystrobin for 9 months (interim sacrifice) or 18 months (terminal sacrifice)

Finding	Sacrifice group	Dietary concentration (ppm)									
		Males					Females				
		0	30	300	1000	2000	0	30	300	1000	2000
Tissues examined	Terminal	50	50	50	50	50	50	50	50	50	49
	Interim	10	10	10	10	10	10	10	10	10	10
Hepatocellular hypertrophy	Terminal	36	37	36	41	44	7	7	7	13	21
	Interim	6	8	8	7	10	2	1		2	5
Single-cell necrosis	Terminal	6	4	7	15	22	5	3	3	6	12
	Interim	1	0	2	2	4	2	0	0	1	5
Necrosis	Terminal	3	3	1	3	1	2	5	5	6	9
	Interim	0	0	0	0	1	1	2	0	0	1
Fatty change	Terminal	32	30	35	36	39	42	33	33	30	25
	Interim	9	10	9	10	9	7	9	8	6	5

From Gerspach (1997a)

nodes were affected (36.2%), compared with incidences of 9 out of 44 (20%) in the concurrent control group; however, the incidences in the treated groups were similar to those at the higher end of the range for historical controls (18/50 or 36.0%). The slightly increased incidences of necrosis in the Harderian gland of males at 2000 ppm were considered by the sponsor to be a consequence of the blood sampling procedure performed in all animals for evaluation of carcinogenicity potential (terminal group). Peritoneal, lymphohistiocytic infiltration was slightly increased in females at 1000 and 2000 ppm. The consulting pathologist stated that peritoneal inflammation is known to occur secondarily to inflammatory processes of abdominal organs. The inflammatory process in other organs or tissues occurred incidentally.

Malignant lymphoma, a systemic neoplasia, occurred in variable incidences in animals of either sex in the control group and in the treated groups (2%, 6%, 4%, 8% and 8% in males and 10%, 18%, 18%, 8% and 22% in females, at 0, 30, 300, 1000 and 2000 ppm, respectively). However, no dose–response relationship was evident and the incidences were well within the range for incidence of malignant lymphoma in the historical control groups (range: males, 2%–9.4%; females, 10%–36.5%). In addition, the concurrent control group had incidences of malignant lymphoma (2% in males and 10% in females) which were closer to the lower end of the range for historical controls. Also, the type and number of organs infiltrated by this tumour varied by chance within a wide range of individual animals. Accordingly, no toxicological relevance was attributed to the increased numbers of infiltrations in certain tissues. Ovarian cysts and pressure atrophy of the brain were present in numbers too small to be of any toxicological relevance. There was no evidence for a treatment-related effect on the incidence of tumour-bearing animals.

The NOAEL was 300 ppm in males and females (equivalent to 39.4 and 35.7 mg/kg bw per day, respectively) on the basis of findings at 1000 ppm—increased absolute and/or relative liver weights in both sexes, increased hepatocellular single cell necrosis in males, and impaired body-weight development (by about 15–30%) in females starting at week 5 and persisting throughout the treatment period. Trifloxystrobin was tested at adequate doses, on the basis of decreased body weight/body-weight gain in females and increased liver pathology findings in both sexes at 2000 ppm, and was found to be not carcinogenic in mice (Gerspach, 1997a).

Rats

In a study conducted in compliance with the principles of GLP (with QA certification), rats were continuously fed diets containing trifloxystrobin at a dose of 0, 50, 250, 750 and 1500 ppm (equal to 1.95, 9.8, 29.7 and 62.2 mg/kg bw per day in males, and 2.2, 11.4, 34.5 and 72.8 mg/kg bw per day in females) for 24 months. Concentrations of trifloxystrobin in the diet were analysed periodically throughout the study. Samples of the diet prepared for the first 4 weeks of the study were analysed for stability (for 5 weeks) and homogeneity. Checks were made twice per day for mortality and daily for clinical signs; body weight and food consumption were recorded weekly for the first 3 months and monthly thereafter; water consumption was recorded monthly. Ophthalmological examinations were conducted at pre-test and at 6, 12, 18 and 24 months and included inspection of the surroundings of the eyes, of sclera, cornea, iris and adaptation of the pupil to light. Haematology (20 of each sex per group), clinical chemistry (10 of each sex per group) and urine analysis (10 of each sex per group) investigations were carried out at weeks 13, 27, 53, 79 and 105. At scheduled sacrifices, the surviving control and treated animals were subjected to detailed necropsy and histopathology. At the scheduled terminal and interim sacrifices, the surviving control and treated animals were subjected to detailed necropsy and histopathology. Organ weights were recorded for adrenal glands, brain, heart, kidneys, liver, ovaries/testes, and spleen.

Towards the end of the study, the incidence of diarrhoea in males at 1500 ppm was increased. No other treatment-related findings were noted. There were no apparent treatment-related deaths. However, significantly more males at 750 and 1500 ppm survived to the end of treatment. The survival rates of animals from the carcinogenicity group were 68% at 750 ppm and 80% at 1500 ppm, compared with 34% in the control group. Better survival was also seen in females at 1500 ppm (80%) in comparison with controls (66%), although statistical significance was not reached. In addition, slightly more male rats given trifloxystrobin at 50 and 250 ppm survived to the end of treatment compared with the control group. However, there was no dose–response relationship and the 24-month survival of males in the control group (17 out of 50, or 34%) was in the low range of historical control values. There were no treatment related ophthalmic changes at any time-point.

Body-weight gain was reduced during most of the study in males and females in the groups receiving trifloxystrobin at 750 or 1500 ppm and in females at 250 ppm (Table 23). Cumulative body-weight gain was generally about 5–6% and 11–17% below that of the controls in males treated at 750 and 1500 ppm, respectively. Statistical significance was reached throughout the entire study period for the group at 1500 ppm, but only during the first 9 months of the study in the group at 750 ppm. Compared with the control group, females at 250, 750 and 1500 ppm had reductions of approximately 5–7%, 9–11% and 17–27%,

Table 23. Cumulative body-weight gain (% of control) in rats fed diets containing trifloxystrobin for 2 years

Week	Dietary concentration (ppm)							
	Males				Females			
	50	250	750	1500	50	250	750	1500
12	99.4	98.6	94.7**	87.0**	99.4	94.7	89.6**	80.9**
51	100.1	100.0	94.8	83.7**	98.9	92.6	89.2**	73.8**
103	106.7	106.1	101.2	93.5	93.2	95.5	91.3	74.5**

From Oishi et al. (1995)
** Statistically significant ($p < 0.01$)

respectively. Statistical significance was reached throughout the entire study period in the group at 1500 ppm, and during most of the time at 750 ppm. The marginal deviations in females at 250 ppm were associated with slightly reduced food intake but never reached statistical significance. As no functional change was observed at this dose, the body-weight effect is considered to be not toxicologically relevant.

Cumulative food consumption throughout treatment in males and females at 1500 ppm was 4% and 8% below that of the control group, respectively (Table 24). A tendency to minimally reduced food intake was also seen in females treated at 250 and 750 ppm (approximately 4% lower overall intake each). Food consumption in the remaining groups was similar to that of the controls. While males in the group receiving the highest dose consumed more water (by 4%) over most of the study compared with control animals, female animals in the same group had a reduced water consumption.

There were minor changes (generally less than ±5% of respective control values) in erythrocyte parameters including increased erythrocyte counts, haemoglobin concentrations, and erythrocyte volume fraction, and lower mean cell volume (MCV), mean corpuscular haemoglobin (MCH), MCHC, and haemoglobin concentration distribution width (HDW) in both sexes at ≥750 ppm, predominantly during the first year of the study. These alterations are considered incidental as there was no clear dose–response relationship or time dependency, and most of the variations were minimal and did not attain statistical significance. Even if the changes are considered to be treatment-related, the magnitude of the effect was too low to be of any toxicological relevance. One male at 50 ppm was found to have a myeloid leukaemia and one female at 750 ppm had a blast cell leukaemia. These pathologies were considered by the sponsor to be of spontaneous origin and not related to treatment.

There were no treatment-related findings in any of the examined blood chemistry parameters. Some intergroup statistically significant differences were seen, including increases in serum concentration of urea (by 11–19%), creatinine (by 7–16%), potassium (by 10%), cholesterol (by 14%), and triglycerides (by 33%) or decreases in concentrations of total bilirubin (by 30%), potassium (by 13–18%), and chloride (by 0.9–2.9%). However, these changes are not considered to be toxicologically relevant owing to the lack of dose-

Table 24. Food consumption (g/animal) in rats fed diets containing trifloxystrobin for 24 months

	Dietary concentration (ppm)				
	0	50	250	750	1500
Cumulative food consumption, total for weeks 1–103 (% of controls):					
Males	17455.9	17710.5	17537.8	17116.2	16785.9
	(NA)	(101.5)	(100.5)	(98.1)	(96.2)
Females	12429.1	12339.7	11893.8	11881.8	11453.7
	(NA)	(99.3)	(95.7)	(95.6)	(92.2)
Time-weighted average food consumption per week (% of controls):					
Males	169.5	171.9	170.3	166.2	163.0
	(NA)	(101.4)	(100.5)	(98.1)	(96.2)
Females	120.7	119.8	115.5	115.5	111.2
	(NA)	(99.3)	(95.7)	(95.6)	(92.1)

From Oishi et al. (1995)
NA, not applicable

dependency, occurrence at a single time-point, the very low magnitude of the changes and/or the changes being in the opposite direction from that considered to be a toxic effect.

The quantitative and qualitative tests on urine did not reveal any evidence for a treatment-related effect. There were a few minor differences that, despite attaining statistical significance, were considered to be incidental, not dose-related, and toxicologically irrelevant.

At interim sacrifice (week 53), the mean carcass weights were reduced in males at 1500 ppm (13.3%) and in females at 750 (9.2%) and 1500 ppm (18.7%). Mean heart : body-weight ratios were increased in females at 750 ppm (14.6%) and 1500 ppm (25.5%). Relative weights of the liver were increased (not statistically significantly) in males at 750 (11.1%) and 1500 ppm (10.0%), while females at 1500 ppm had statistically significantly increased mean relative weights of the liver (23.9%) and kidney (20.2%).

At terminal sacrifice, the mean carcass weight of females at 1500 ppm was still significantly lower (16.5%) than that of the control group. Some organs from males and females at 1500 ppm had reduced absolute weights (by about 7–20%), probably commensurate with reduced body-weight development. Significant increases were seen in the mean relative weights of the heart (12%), liver (8.8%), and kidneys (12.4%) in females at 1500 ppm. Increased relative testes weights in the animals at 1500 ppm (21.7%) was attributed to the occurrence of fluid contents in the albugineous tunica of some animals, as was noted at necropsy. Since there were no microscopic correlates, these observations are most likely not treatment-related.

At interim necropsy, there were no macroscopic findings indicating an effect of treatment. At terminal necropsy, there were decreased incidences of masses on body surfaces and decreased incidence of enlarged pituitary gland in males and females of the groups at 1500 and 750 ppm. These observations reflected a decrease in tumour incidences and are not toxicologically relevant.

At interim necropsy, there were no treatment-related, non-neoplastic histopathology findings except for a slightly decreased incidence of fatty liver and pancreas in females at 750 and 1500 ppm. Decreased incidences of fatty liver and pancreas became more prominent at terminal necropsy. These changes possibly reflect the low mean body weight, especially in the females at 1500 ppm.

Owing to significantly increased survival in the treated groups, several age-related spontaneous histopathology findings, which tend to occur in geriatric animals, were seen at higher incidences in treated animals especially among the males at 1500 ppm. Such increased incidences, however, probably reflected the age difference rather than being a direct effect of treatment. In particular, when adjusted for time-dependence, statistical analysis revealed no significant differences, except for the findings of angiomatous hyperplasia in the mesenteric lymph node and of developmental cysts in the pituitary gland. The consulting pathologist stated that these findings were not considered to be treatment-related because angiomatous hyperplasia of the mesenteric lymph node is a characteristic spontaneous age-related finding in rats, and developmental cysts of the pituitary gland represent a developmental change already present in the animals before the beginning of treatment.

Overall, there were no apparent treatment-related neoplastic findings. At interim necropsy, the numbers of observed tumours were low and not affected by treatment. At

terminal necropsy, the overall incidence of tumours, including several specific tumour types, were decreased in a dose-related manner. The decreased incidence of tumours in general probably reflects the development of lower body weight in treated animals when compared with controls, and may not be a direct effect of trifloxystrobin.

Certain tumours, however, appeared to be increased dose-dependently (Table 25). The sponsor explained that the apparent increases were caused by the higher number of survivors to termination in the groups at 750 and 1500 ppm and that the tumour types and incidences were comparable with findings normally occurring in ageing rats. According to the sponsor, the increased incidences of benign adrenal medullary tumours and haemangiomas of the mesenteric lymph node in males at 1500 ppm are a result of random distribution, in view of the common occurrence of these tumour types in ageing male rats (Losco & Harleman, 1992). The sponsor also argued that the microscopic features of the findings in the study were characteristic of spontaneous lesions based on irregularly sized blood-filled spaces lined by elongated cells with oval nuclei, with solid sheets of oval cells in larger tumours. According to the consulting pathologist, the origin of these lesions is unknown and some authors do not consider them to be neoplastic and prefer to characterize them as "mesenteric disease". The observed incidence in the males at 1500 ppm was 5 out of 49 (10.2%), which is above the maximum value for historical controls of 4 out of 59 (6.8%). However, the consulting pathologist stated that it is important to note that all five cases in the group at 1500 ppm were observed in animals surviving until terminal sacrifice and this lesion is age-related, and consequently the relative incidence of this finding in the survivors is the more appropriate index for comparison. Following this approach, the sponsor argues that the finding in this study was not treatment-related since the relative incidence of 5 out of 40 (12.5%) in the study was comparable to the maximum for historical controls of 3 out of 26 (11.5%).

There was an indication that in the treated animals a few tumours were observed earlier than in the control groups. For example, an adenocarcinoma of the mammary gland was noted at week 16, and a malignant astrocytoma of the brain in a female was noted at week 26, both of these in the group receiving trifloxystrobin at 1500 ppm. The sponsor placed no toxicological relevance on the occurrence of these two neoplasms owing to their

Table 25. Selected tumours occurring at apparently increased incidences in male rats fed diets containing trifloxystrobin for 24 months

	Dietary concentration (ppm)				
	0	50	250	750	1500
Adrenal medullary tumour					
Organs examined	50	50	50	50	50
Organs with benign tumour	0	2	3	3	5
Organs with malignant tumour	1	1	0	1	0
Organs with either tumour	1	3	3	4	5
Mesenteric lymph node: haemangioma					
Organs examined	49	50	49	49	49
Organs affected	0	1	1	2	5
Organs examined in survivors	17	25	23	34	40
Organs affected in survivors	0	0	1	2	5
% affected in survivors	0	0	4.3	5.9	12.5

From Oishi et al. (1995)

low incidence and because the general occurrence of neoplasms was decreased in a dose-related manner. Moreover, a spontaneous early occurrence of mammary adenocarcinoma in a female Sprague-Dawley rat aged 10 weeks has been reported (Oishi et al., 1995).

A variety of other non-neoplastic and neoplastic changes in the study were marginally increased or were considered to be findings that are known to occur spontaneously in laboratory rats.

The NOAEL was 250 ppm for both sexes (corresponding to 9.8 and 11.4 mg/kg bw per day in males and females, respectively), based on the reduction in body weight and body-weight gain at the next higher dose. Dosing was considered adequate for testing for carcinogenicity in rats on the basis of decreased body-weight gain in males (by 11–17%) and females (by 17–27%) at 1500 ppm, the highest dose, in addition to decreased food consumption in females (by 7.9%) at the same dose. Trifloxystrobin was not carcinogenic in rats (Gerspach, 1997b).

2.4 Genotoxicity

Trifloxystrobin (purity, 96.4%) was evaluated for potential genotoxicity in an adequate range of assays performed in vitro and in vivo (Table 26). The tests performed in vitro included tests for mutagenicity in bacterial and mammalian cells, for chromosome damage (clastogenicity) and for unscheduled DNA synthesis. In all tests carried out in vitro, trifloxystrobin was tested at adequate concentrations resulting in cytotoxicity and/or precipitation. With the exception of the test for forward gene mutation, the results of these studies demonstrated the absence of a genotoxic effect (Table 26). In the teste for forward gene

Table 26. Results of studies of genotoxicity with trifloxystrobin

End-point	Test object	Concentration	Purity (%)	Result	Reference
In vitro					
Reverse mutation[a,b]	*S. typhimurium* TA98, TA100, TA102, TA1535, TA1537; *E. coli* WP2 uvrA	62–5000 µg/plate ± S9; in DMSO	96.4	Negative	Hertner (1994a)
Forward mutation[c,d]	Chinese hamster V79 lung cells	11.11–833.5 µg/ml +S9; 0.14–833.5 µg/ml –S9; both in DMSO	96.4	Positive at cytotoxic doses Equivocal	Hertner (1995a)
Chromosomal aberrations[c,e]	Chinese hamster ovary (CHO-K1)	0.78–200 µg/ml +S9 0.049–200 µg/ml –S9; in DMSO	96.4	Negative Negative	Hertner (1994b)
Unscheduled DNA synthesis[f,g]	Rat hepatocytes (male Sprague-Dawley)	0.39–400 µg/ml 0.39–50 µg/ml; in DMSO	96.4	Negative Negative	Hertner (1995b)
In vivo					
Chromosomal aberrations[h]	Mouse (Tif:MAG)	1250–5000 mg/kg bw; in carboxymethyl cellulose	96.4	Negative	Hertner (1995c)

S9, 9000 × *g* supernatant of liver of rats induced with Aroclor 1254; DMSO, dimethylsulfoxide

[a] Test in triplicate; positive control included; GLP and QA statements provided

[b] Cytotoxicity was not seen at 5000 µg/plate with or without S9; test material precipitated at or above 1250 µg/plate

[c] Test in duplicate; positive control included; GLP and QA statements provided

[d] Cytotoxicity observed at doses of >0.41 µg/ml without S9 (by 94–95%) and >6.5 µg/ml with S9 (by 6.4–92.4%); test material precipitated at or above 50 µg/ml without S9 and at or above 150 µg/ml with S9

[e] Cytotoxicity observed at doses of >3.125 µg/ml without S9 and >100 µg/ml with S9

[f] Two independent trials were done; positive control included; GLP and QA statements provided

[g] Cytotoxicity observed at doses of >50 µg/ml; test material precipitated at or above 25 µg/ml

[h] Aberration tested in five animals of each sex per group for each sacrifice time; positive control included; absorption or transport of compound to bone marrow was not tested but limit dose was achieved; GLP and QA statements provided

mutation in Chinese hamster V79 cells, there were slight statistically significant increases in mutant frequencies at cytotoxic doses in the presence of metabolic activation. Results were equivocal in the absence of metabolic activation. Trifloxystrobin was also assessed for induction of micronucleus formation in mice. The result of this study showed that trifloxystrobin does not exhibit a chromosome damaging potential in vivo.

Despite the equivocal mutagenicity findings in the mammalian test system, the overall in findings vitro and in vivo support the conclusion that trifloxystrobin is not likely to be mutagenic or genotoxic.

2.5 Reproductive toxicity

(a) Multigeneration studies

Rats

In a study conducted in compliance with the principles of GLP with QA certification, male and female Sprague-Dawley (Tif:RAIf) rats were given diets containing trifloxystrobin (purity, 96.4%) at a nominal concentration of 0, 50, 750 or 1500 ppm continuously over two successive generations (F_0 and F_1). After 10 weeks of dietary exposure to trifloxystrobin before mating, animals were paired 1:1 within each dose group (30 of each sex per group) until there was evidence of positive mating or for 19 days, whichever occurred first. Dams were allowed to litter and suckle their pups naturally. On postnatal day 4, litters were culled to four male and four female pups. After weaning of the F_1 pups, the F_0 parental animals were remated to produce a second set of litters. The F_1 generation was selected from the first litters of the F_0 generation. The animals were checked daily for mortality and clinical signs (twice per day if signs were observed); body weights were recorded weekly; food consumption was determined weekly (except during cohabitation for mating) and were reported as daily means. Also, parental mating, fertility, and gestation indices were determined.

Signs of difficult or prolonged parturition were recorded. All animals were necropsied and subjected to a complete macroscopic pathological examination with special attention to the reproductive system. Organ weights from parental animals were recorded for adrenal glands, brain, kidneys, liver, ovaries/testes, spleen, and thymus. Full histopathological examination was performed on selected organs/tissues, including vagina, uterus, ovaries, testes, epididymides, seminal vesicles, prostate, pituitary gland, liver, pancreas, and all gross lesions, from all control and at the highest dose F_0 and F_1 animals that were selected for mating. The liver, spleen, and kidneys from all males and females of both F_0 and F_1 generations were wighed and the same organs were examined histopathologically.

The number of viable and stillborn pups was determined on postnatal day 0. Mortality and clinical signs were checked daily from postnatal day (twice per day if signs observed); body weights were recorded on postnatal days 0, 4, 7, 17 and 21. Righting reflex was tested from postnatal day 2 to 100% occurrence. Eye opening was examined daily from postnatal day 14 to 100% occurrence. Live birth, viability, and lactation indices were also determined.

The F_{1A} pups that were not selected for mating, in addition to F_{1B} and F_2 pups, were killed on or shortly after weaning of the last litter of that mating. All these animals, as well as all culled pups and those found dead or killed in a moribund condition, were subjected

to gross necropsy, which consisted of macroscopic examination of the body, limbs, and organs of the thoracic and abdominal cavities, with special attention to the reproductive system.

Trifloxystrobin was found to be homogeneously distributed and stable in the diet for at least 5 weeks at room temperature. In males/females of the groups at 50, 750 and 1500 ppm, the overall mean intake of test substance was 3.8/4.1, 55.3/58.0 and 110.6/123.1 mg/kg bw per day for the F_0 generation and 4.2/4.4, 65.5/67.0, and 143.0/146.0 mg/kg bw per day for the F_1 generation, respectively.

No treatment-related mortality or clinical signs were seen in parental F_0 animals. At 1500 ppm, food consumption was reduced in both sexes, resulting in a retarded body-weight development from the start of the dosing period with final body weights being 8.6% and 7.1% below that of males and females in the control groups, respectively. A slightly reduced overall body-weight gain was also observed in females at 750 ppm during first gestation (days 7–14, 3.8%; days 14–21, 12.4%), which was also associated with a slightly reduced food consumption (days 7–14, 3.3%; days 14–21, 3.6%). Body-weight gain during the first lactation period was lowered at 750 (27.2%) and 1500 ppm (21.8%), but was significantly increased during the second lactation period at 750 (days 0–7, 141%; days 0–21, 290%) and 1500 ppm (days 0–7, 991%; days 0–21, 355%).

The number of animals mating and the number of females becoming pregnant was not affected by treatment during either mating period for the F_0 generation. Also, there were no treatment-related effects on any of the gestation or parturition indices at the first or second mating. Viability and lactation indices for F_{1A} and F_{1B} litters were comparable in all groups, and the sex ratio among F_{1A} and F_{1B} pups was not changed by treatment with trifloxystrobin.

At 1500 ppm, absolute weights of the spleen in males and adrenals in females were significantly lowered by 13.4% and 8.0%, respectively. Also, relative organ weights were significantly increased in most cases in F_0 males (14.9%, 15.5%, 10.7% and 11.0% at 1500 ppm in liver, kidneys, testes and brain, respectively) and F_0 females (11.1%, 9.7%, 14.5% and 8.4% at 1500 ppm in liver, kidneys, ovaries and brain, respectively). At 750 ppm, a slight statistically significant increase in the relative weights of the liver and kidney (each by 5.7%) in males and liver (by 5.7%) and ovaries (by 12.5%) in females was considered to be of little or no toxicological significance.

Necropsy of F_0 parents revealed no treatment-related macroscopic changes and no histopathological findings in the reproductive organs. Some of the histopathology findings in F_0 parental animals are shown in Table 27. There was increased centrilobular hepatocyte hypertrophy (minimal to moderate) among F_0 males and females at 1500 ppm (Table 27). Also, there was increased incidence of minimal pigmentation of renal tubules in males and females at 1500 ppm and in males at 750 ppm. Decreased incidence of splenic haemosiderosis (pigmentation disturbances) was observed in males and females at 750 and 1500 ppm. Splenic haemosiderosis might correlate with increased iron overload (Klaassen, 1996) or possibly with erythrocyte turnover. However, there was no evidence of decreased erythrocyte turnover to account for the observed decrease in haemosiderosis. Hence, while possibly treatment-related, this observation may not be adverse. There were no treatment-related histopathology findings in the reproductive systems of males or females.

Table 27. Summary of incidence of histopathology findings in F_0 parental rats given diets containing trifloxystrobin

Finding	Dietary concentration (ppm)							
	Males				Females			
	0	50	750	1500	0	50	750	1500
Number of tissues rxamined	30	30	30	30	30	30	30	30
Kidney: pigmentation	1	0	4	7	0	0	0	3
Liver: hepatocellular hypertrophy	3	1	4	10	1	0	1	5
Spleen: haemosiderosis	17	20	12	9	23	22	15	8

From Khalil (1997)

There were no clinical signs in F_{1A} and F_{1B} pups that could be considered to be related to treatment of the dams. Mean pup weights at birth were similar in all groups of F_{1A} and F_{1B} generation. Body-weight development of male and female pups at 750 and 1500 ppm was significantly reduced during lactation to a similar extent. At weaning on postnatal day 21, mean body weights of combined male and female F_{1A} pups at 1500 and 750 ppm were 28.4% and 9.3% below values for the controls, respectively. The respective values for the F_{1B} generation were 27.2% and 10.8% below values for the controls. For F_{1A} and F_{1B} pups at 1500 ppm, mean values for eye opening were delayed by 0.7 and 0.6 days, respectively, compared with control animals. This developmental delay is consistent with the retarded body-weight development. Necropsy of F_{1A} and F_{1B} pups revealed no treatment-related macroscopic findings.

In F_1 parental animals, there were no treatment-related mortality or clinical signs. One male at 750 ppm was sacrificed in a moribund condition on day 85. Piloerection, reduced activity, and respiratory sounds had been noted for 1 day, but no related macroscopic changes were observed at necropsy. Incidental clinical signs observed occasionally included palpable masses, hair loss, various wounds and/or crust/scurf, swelling and chromodacryorrhea.

In F_1 parental animals, food consumption was significantly reduced before day 50 in males treated at 1500 ppm. The decrease ranged from 20.4% for days 1–8 to 8.4% for days 43–50; values were similar to those of controls after day 50 of the study. Food consumption was also significantly reduced in F_1 parental females treated at 750 (ranging from 9.0% for days 1–8 to 6.2% for days 57–64; values were similar to those of the controls after day 64) and 1500 ppm (ranging from 20.6% for days 1–8 to 7.3% for days 57–64) throughout the F_1 generation. Food consumption in the remaning parental groups was similar to that of the control groups throughout the F_1 generation.

Throughout the F_1 generation, body weights in both sexes at 750 and 1500 ppm remained significantly lower than those of the controls. At 750 ppm, the mean decrease in body weight in males was 7.4% over 19 observations; in females, the mean decreases were 9.4% over 12 observations before mating, 8.4% over four observations during gestation, and 7.3% over four observations during lactation. At 1500 ppm, the mean decrease in males was 17.5% over 19 observations; in females, the mean decreases were 18.8% over 12 observations before mating, 15.7% over four observations during gestation, and 14.4% over four observations during lactation. In F_1 males, body-weight gain was decreased for days 1–8 at 750 (by 7.7%) and 1500 ppm (by 16.9%), and for days 8–15 at 1500 ppm (by 13.9%), but

was increased for days 22–29, 68–71 and 71–78 at 1500 ppm (by 14.3%, 554% and 227%, respectively). In F_1 females, body-weight gain was decreased at 1500 ppm before mating for days 68–71 (by 147.8%) and during gestation for days 0–21 at 750 ppm (by 8.2%) and at 1500 ppm (by 19.4%). However, body-weight gain in F_1 females was increased during lacatation for days 0–21 at 750 ppm (by 192%) and at 1500 ppm (by 299%). The sponsor considered that the increased body-weight gain during lactation was related to the marked retardation in body-weight development of the pups in this group, resulting in them being suckled longer and weaned later than usual by the dams.

There were no treatment-related effects on the number of animals mating, the number of females becoming pregnant or on the mean precoital time. Five males (one in the control group, one at 50 ppm, two at 750 ppm and one at 1500 ppm) failed to mate. One mated female was not pregnant. Also, there were no effects on the duration of gestation (from 22.0 to 22.1 days in all groups) or parturition with a total of 29, 28, 28 and 29 pregnant females giving birth to live young in the groups at 0, 50, 750 and 1500 ppm, respectively.

Both the viability index (percentage of pups surviving postnatal days 0–4) and the lactation index (percentage of pups surviving postnatal days 4–21) were similar to those of the control group. Dams (28, 28, 28 and 29 at 0, 50, 750 and 1500 ppm) successfully reared their litters to weaning on postnatal day 21. The sex ratios of the F_2 pups on postnatal days 0 and 21 were similar in all groups.

In F_1 adult animals, both sexes at 1500 ppm group had significantly lower body weights at sacrifice (15.0% and 16.6% in males and females, respectively). In the same group, males had significantly decreased absolute weights of the spleen (by 11.5%) and brain (by 5.7%), while females had reduced absolute weights of kidneys (by 9.1%) and brain (by 3.5%). Compared with controls, relative organ weights were significantly increased in most cases in both the F_1 males (12.4, 11.3, 16.7, 18.5 and 10.8% at 1500 ppm for liver, kidneys, testes, adrenals and brain, respectively) and F_1 females (12.8%, 9.2%, 16.2%, 19.8% and 15.8% at 1500 ppm for liver, kidneys, ovaries, thymus and brain, respectively). At 750 ppm, absolute weights of the brain in males were significantly lowered (4.1%), while, in females, the absolute weights of liver and kidney were decreased (by 10.8% and 9.1%, respectively), but that of the thymus was increased (by 12.5%). The effects on absolute weights of the thymus in females at 750 ppm group were considered to be incidental by the consulting pathologist due to the lack of dose-dependency. At 50 ppm, relative weights of the thymus were significantly increased (11.8%) in females. The relative increase in weight of the thymus is not considered toxicologically to be significant since there were no corroborating histopathology findings in the thymus.

Microscopic findings in the F_1 parental animals were unremarkable but similar to those seen in F_0 parental animals. In the liver, there were increased incidences of minimal to moderate centrilobular hepatocyte hypertrophy in males and females at 750 ppm (14/30 and 7 out of 30, respectively) and at 1500 ppm (24 out of 30 and 9 out of 30, respectively) compared with 1 out of 30 and none out of 30 incidences in males and females in the control group, respectively. Microscopic examination of the spleen in males and females showed decreased incidence of splenic haemosiderosis from 14 out of 30 and 23 out of 30 in the control group to 5 out of 30 and 17 out of 30 at 750 ppm, and 2 out of 30 and 14 out of 30 at 1500 ppm, respectively. As noted for the F_0 parents, splenic haemosiderosis may correlate with turnover of erythrocytes such that decreased splenic hemosiderosis may be caused by decreased turnover of erythrocytes. However, a decrease in erythrocyte turnover was not

observed in any of the studies of toxicity with trifloxystrobin. Although possibly treatment-related, the findings of decreased splenic haemosiderosis are considered not to be adverse.

There were no treatment-related histopathology findings in the reproductive systems of animals of both sexes.

In the F_2 pups, there were no treatment-related clinical signs and the mean pup weights at birth were similar in all groups. During lactation, body weights of the F_2 pups at 750 and 1500 ppm were significantly decreased on lactation days 7 (males: by 9.9% and 15.9%; females: by 8.2% and 17.0%, respectively), 14 (males: by 12.3% and 21.3%; females: 9.2% and 21.8%, respectively), and 21 (males: by 16.7% and 28.5%; females: 12.3% and 27.6%, respectively). Body-weight gains were also significantly reduced in F_2 pups in the same respective groups on lactation days 0–4 (females: by 12.9% and 22.6%), 4–7 (males: by 14.3% and 28.6%; females: by 12.5% and 30.4%), 7–14 (males: by 15.9% and 27.2%; females: by 10.3% and 27.4%) and 14–24 (males: by 22.4% and 37.9%; females: by 16.6% and 35.5%). At 50 ppm, mean pup weights and mean pup body-weight gain were similar to that of the control group after culling on postnatal day 4 until to weaning on postnatal day 21.

For F_2 pups at 1500 ppm, mean values for eye opening were delayed by 0.7 days compared with that of control animals, which is consistent with the retarded body-weight development. Values for eye opening were similar to that of the control group for all other doses. Mean values for surface righting were similar for all groups.

There were no treatment-related macroscopic necropsy changes and no histopathology findings in the reproductive organs of the pups. Conjoined twins with exencephaly were seen in one stillborn pup at 750 ppm, but there was no evidence that this finding was treatment-related.

The NOAEL for parental toxicity was 50 ppm, equal to 3.8 mg/kg bw per day, on the basis of reduced body weights and body-weight gains, reduced food consumption, and histopathology observations in liver and kidneys. The NOAEL for offspring toxicity was 50 ppm, equal to 3.8 mg/kg bw per day, on the basis of decreased pup body weights during lactation. The NOAEL for reproductive toxicity was 1500 ppm, equal to 110.6 mg/kg bw per day, the highest dose tested (Khalil, 1997).

(b) Developmental toxicity

Rats

In a study conducted in compliance with the principles of GLP (with QA certification), groups of 24 pregnant Sprague-Dawley (Tif:RAIf) rats were given trifloxystrobin (purity, 96.4%; in 0.5% aqueous sodium carboxymethylcellulose) at a dose of 0, 10, 100 or 1000 mg/kg bw per day by oral gavage from day 6 to day 15 of gestation. Dams were sacrificed on day 21 of gestation and fetuses were removed. Mortality, clinical signs, and body weights were recorded daily; food intake was determined on days 6, 11, 16 and 21 and daily consumption was calculated. After removal from the uterus, fetuses were numbered, weighed, sexed and examined for external malformations. After killing by subcutaneous injection of barbiturate, fetuses were processed for visceral or skeletal examination (at a ratio of approximately 1:1). Food, water and the housing environment were controlled and monitored.

Dose selection for this main study was based on a preliminary dose-range finding study in which four groups of seven mated virgin female albino Sprague-Dawley rats (Tif:RAIf) aged 2 months were given trifloxystrobin (in sodium carboxymethylcellulose) at a dose of 0, 10, 100 or 1000 mg/kg bw per day from day 6 to day 15 of gestation, inclusive. No treatment-related effects were noted in clinical signs, body weights and body-weight gains, postimplantation loss, fetal weights, mean gravid uterine weights, carcass weights, maternal necropsy or external fetal observations. There was a slight reduction in food consumption at 1000 mg/kg per day (Fitzgerald, 1993).

In the main study, all dams survived until terminal sacrifice. One dam in the group at 10 mg/kg bw per day and one in the group at 100 mg/kg bw per day had hair loss starting on day 11 and 13, and one dam in the group at 1000 mg/kg bw per day had an ear wound on days 13 and 14 and then ear crust/scurf until necropsy. Haemorrhagic discharge in the perineal area was seen in one animal at 100 mg/kg bw per day and six animals at 1000 mg/kg bw per day. This finding was observed for 1 day only, and all these animals had normal pregnancies. Three of these animals had no resorptions and four animals had one to four resorptions. These findings were not regarded as treatment-related by the sponsor. At 1000 mg/kg bw per day, mean absolute maternal body weight was consistently less than that of the controls; this difference were statistically significant on days 8 (by 3.7%) and 16 (by 5.3%), but body weight was fully recovered thereafter. Mean body-weight values for the groups at the lowest dose remained comparable with control values throughout the study. There were statistically significant reductions in mean maternal body-weight gains at 1000 mg/kg bw per day during the entire dosing period (days 6–16 of gestation, 21%) as well as within the dosing period (e.g. days 6–11 of gestation, 37.2%; days 11–16 of gestation, 20.9%). Also, the corrected maternal body-weight gain for the dosing period plus the post-dosing period (i.e. net body-weight change from day 6 of gestation, which equals the carcass weight minus body weight on day 6) was statistically significantly decreased at 100 and 1000 mg/kg bw per day (by 17.8% and 32.5%, respectively). During the post-treatment period (days 16–21 of gestation), there was a slight compensatory increase in body-weight gain noted at 1000 and 100 mg/kg bw per day compared with controls (by 4.1% and 2.1, respectively). Food consumption was significantly reduced at 100 mg/kg bw per day (days 6–11 of gestation, 7.6%; days 11–16 of gestation, 7.7%) and 1000 mg/kg bw per day (days 6–11 of gestation, 29.6%; days 11–16 of gestation, 15.5%) during the treatment period.

Of the 24 mated animals per group, one, two, four and two were not pregnant in the control group, and at 10, 100 and 1000 mg/kg bw per day, respectively. The number of pregnant animals with viable fetuses at scheduled necropsy was 23, 22, 20 and 22, respectively. Preimplantation losses, number of implantation sites, and early and late postimplantation losses were comparable between all groups. No dead or aborted fetuses were noted. Numbers of live fetuses/litter and fetal weights were not affected by treatment.

There was a reduction in maternal carcass weight at 1000 mg/kg bw per day (by 6.0%) as well as in mean net body-weight change from day 6 in the groups receiving trifloxystrobin at 100 (by 17.8%) and 1000 mg/kg bw per day (by 32.5%) relative to the control group (also discussed above). The reduction in mean net body weight was dose-related. Necropsy of all maternal animals showed no macroscopic changes. External examination of fetuses revealed no treatment-related abnormalities. An umbilical hernia was found in a control fetus and a generalized oedema in one fetus at 10 mg/kg bw per day. Upon visceral examination, the same two animals showed an umbilical hernia and pulmonary hyperplasia, respectively (Table 28). Additionally, renal pelvic dilatation was found in three fetuses of

Table 28. Summary of observations in rat fetal viscera

	Dose (mg/kg bw per day)			
	0	10	100	1000
Total fetuses examined/litters examined	149/23	135/22	139/20	146/22
Umbilical hernia	1/1	ND	ND	ND
Enlarged thymus	3/3	3/1	3/3	11*/7
Pulmonary hyperplasia	ND	1/1	ND	ND
Accessory lobulets in liver	1/1	2/2	1/1	2/2
Renal pelvic dilitation	3/3	1/1	ND	1/1
Total visceral observations	8/8	7/4	4/4	14/10

ND, none detected
* Chi-squared test + Fisher's Exact test, $p < 0.05$

the control group and in one fetus each at 10 and 1000 mg/kg bw per day. One to two fetuses in each group had accessory liver lobulets. None of these effects were considered to be treatment-related by the consulting pathologist. Enlarged thymus was seen in three, three, three and eleven fetuses in the control group and at 10, 100 and 1000 mg/kg bw per day, respectively. The increased incidence of enlarged thymus at 1000 mg/kg bw per day (7.5% of fetuses/32% of litters) exceeded both the mean (0.7%/4.0%) and maximum (6.0%/29.2%) fetal/litter incidences for historical controls and was likely to be treatment-related.

No skeletal malformations were noted. The incidence of skeletal anomalies included fused or asymetric sternebrae, irregular ossification of cranial bones, poor ossification of the fifth metacarpal, additional cervical vertebral arches, and bipartite thoracic vertebral centres. Skeletal variations consisted of poor or absent ossification of sternebrae, calcaneus first metatarsal, cervical and thoracic vertebral centres, ribs and phalanges, bipartite or dumbell-shaped cervical or thoracic vertebral centres, and shortened thirteenth rib. None of the skeletal abnormalities were considered to be treatment-related.

Trifloxystrobin was not teratogenic in rats. The NOAEL for maternal toxicity was 10 mg/kg bw per day on the basis of reduced body-weight gain and food consumption at 100 mg/kg bw per day. The NOAEL for embryotoxicity was 100 mg/kg bw per day on the basis of increased incidence of enlarged thymus at 1000 mg/kg bw per day. There was no evidence of teratogenic potential (Khalil, 1995).

Rabbits

In a study conducted in compliance with the principles of GLP (with QA certification), groups of 19 pregnant Russian (Chbb : HM) rabbits were given trifloxystrobin (purity, 96.4%; in 0.5% aqueous sodium carboxymethylcellulose) at a dose of 0, 10, 50, 250 or 500 mg/kg bw per day by oral gavage from day 7 to day 19 of gestation. Dams were sacrificed on day 29 of gestation and fetuses were removed. Mortality, clinical signs, and body weights were recorded daily; food intake was determined on days 4, 7, 12, 16, 20, 24 and 29 and daily food consumption was calculated. Dams were killed on day 29 and the following observations were recorded at necropsy: macroscopic pathological examination of the main organs of the thoracic and abdominal cavities, in particular the genitals; number of corpora lutea in each ovary; weight of the uterus including contents; uterine contents for dams at scheduled necropsy (number and location of live and dead fetuses, number and location of early and late embryonic/fetal losses, total postimplantation and/or abortion sites) and for dams sacrificed or dying before scheduled necropsy (number and location of implantation and/or abortion sites). After removal from the uterus, fetuses were numbered,

weighed, sexed and examined for external malformations. After killing by subcutaneous barbiturate injection, fetuses were processed for visceral or skeletal examination (at a ratio of approximately 1 : 1). Food, water and the housing environment were controlled and monitored.

Dose selection for this main study was based on a preliminary dose-range finding study in which five groups of five artificially inseminated virgin female rabbits (Russian Chbb : HM) aged 3 months were given trifloxystrobin at a dose of 0, 20, 100, 500 or 1000 mg/kg bw per day (in sodium carboxymethylcellulose) from days 7 to 19 of gestation, inclusive. No animals died. There was reduced activity in all animals at the highest dose and two animals exhibited haemorrhagic discharge in the perineal area on several occasions. At 500 mg/kg bw per day or above, animals had reduced body weight, body-weight gain, and food consumption; reduced food consumption was also noted in the group at 100 mg/kg bw per day. All animals at the highest dose in addition to one animal at 500 mg/kg bw per day had total resorptions. At 500 mg/kg bw per day, animals had reduced gravid uterine weights, the number of fetuses was decreased, and postimplantation loss was increased. Mean fetal weight was also reduced in the group at 500 mg/kg bw per day. There were no treatment related effects in the number of corpora lutea, implantation sites, preimplantation loss, external fetal observations, or findings on maternal necropsy (Khalil, 1994a).

In the main study, there was no treatment-related mortality or clinical signs. One dam at 50 mg/kg bw per day died spontaneously on day 27 of gestation without having exhibited any clinical signs before death. At necropsy, this animal was found to have haemorrhagic contents of the uterus. Other incidental findings included hair loss and a palpable mass in the head of one animal at 10 mg/kg bw per day.

Maternal body weights were retarded in the animals at 250 (day 12, 5.4%; day 21, 5.4%) and 500 mg/kg bw per day (day 12, 5%; day 21, 6.3%) from the start of the dosing period until day 21. Body weights were unaffected by treatment at 10 and 50 mg/kg bw per day. During the treatment period (days 7–19 of gestation), there was a significant net weight loss of 83 g and 152 g (compared with controls) in the groups receiving trifloxystrobin at a dose of 250 and 500 mg/kg per day, respectively (Table 29).

As shown in Table 30, food consumption was also significantly reduced during the treatment period, namely during days 7–12, 12–16, and 16–20 by 65.5%, 47.2%, and 34.8%,

Table 29. Maternal body-weight gain in a study of developmental toxicity in rabbits

Days	Dose (mg/kg bw per day)				
	0	10	50	250	500
0–4	24	25	43	30	36
4–7	8	−9	−8	−13	−8
7–12	2	10	−22	−120**	−150**
12–16	59	55	54	55	21**
16–20	2	−5	2	−18	−23
20–24	25	31	36	128**	146**
24–29	83	97	76	68	75
7–20	64	59	34	−83**	−152**

From Khalil (1994b)
** Statistically significant ($p < 0.01$)

Table 30. Food consumption (g/animal per day) in a study of developmental toxicity in rabbits

Days	Dose (mg/kg bw per day)				
	0	10	50	250	500
0–4	127.0 ± 27.6	122.6 ± 18.1	130.8 ± 19.6	123.9 ± 15.8	136.0 ± 48.2
4–7	121.8 ± 25.8	109.3 ± 19.8	123.4 ± 17.3	112.0 ± 21.0	116.6 ± 17.0
7–12	109.5 ± 22.0	103.9 ± 16.5	93.7 ± 16.0	37.8** ± 9.1	37.6** ± 5.2
12–16	106.7 ± 24.5	83.5 ± 31.6	91.9 ± 26.2	56.3** ± 16.2	52.0** ± 9.2
16–20	98.8 ± 18.9	91.4 ± 26.3	102.3 ± 18.0	64.4** ± 26.4	58.7** ± 26.4
20–24	92.8 ± 26.5	93.6 ± 30.6	107.2 ± 18.9	122.4 ± 39.0	109.4 ± 34.5
24–29	90.6 ± 23.4	90.6 ± 25.8	98.5 ± 21.3	107.4 ± 27.6	118.1** ± 25.0

From Khalil (1994b)
** $p < 0.01$ by ANOVA + Dunnett test

Table 31. Food efficiency[a] (%) in a study of developmental toxicity in rabbits

Days	Dose (mg/kg bw per day)				
	0	10	50	250	500
0–7	3.7	2.1	3.9	2.0	3.1
7–20	4.7	4.6	2.7	NV	NV
20–29	13.1	15.4	12.2	19.2	21.4
7–29	9.9	9.1	6.7	6.7	4.1
0–29	7.8	7.1	5.9	5.2	3.8

From Khalil (1994b)
NV, negative value for food efficiency because of loss in body-weight gain during the dosing period
[a] Food efficiency was calculated as % body-weight change relative to food intake during a specified period

respectively, at 250 mg/kg bw per day and by 65.7%, 51.5% and 40.6%, respectively, at 500 mg/kg bw per day. A recovery in food consumption was noted during the post-dosing period (after day 20). At 10 and 50 mg/kg bw per day, food consumption was comparable to that of the control group throughout the study.

Food efficiency (calculated by reviewer from data on means) was also reduced during the dosing period for the groups at 250 and 500 mg/kg bw per day (Table 31).

There were no dead or aborted fetuses in any of the groups, and the number of live fetuses per litter and fetal weights (both sexes) were unaffected by treatment. The numbers of corpora lutea, preimplantation losses, numbers of implantation sites, and postimplantation losses were comparable between groups.

Gravid uterus weights and carcass weight did not differ significantly between groups, although carcass-weight change from day 7 was slightly but not significantly reduced (by 35.3%). One dam in the control group had hypoplasia of the left uterus horn, one animal at 10 mg/kg per day had a palpable mass on the head and one animal at 50 mg/kg bw per day had haemorrhagic contents of the uterus. These findings are not considered to be related to treatment. There were no other remarkable observations at maternal necropsy.

Fetal external and visceral examinations revealed no treatment-related abnormalities. A single fetus in the group at 10 mg/kg bw per day showed craniocele (brain hernia may be

associated with a skull defect). Several limb and gastric malformations were seen in only one animal at 250 mg/kg bw per day, but were considered to be unrelated to treatment because of lack of dose-dependency. Forelimb position anomaly was evenly distributed among all treated groups and was within the range of incidence for historical controls; therefore, it is not considered to be a treatment-related malformation.

Findings in the fetal viscera included aplasia (lack of development) of the gall bladder occurred in one fetus at 50 mg/kg bw per day and in two at 500 mg/kg bw per day; in addition, one to two small gall bladders were found in all treated groups. These findings are considered to be developmental variations that are unrelated to treatment since no statistical significance or dose-dependency were found and since a variety of gall bladder findings were present in the data for historical controls.

Skeletal malformations were observed in one fetus at 10 mg/kg bw per day (reduced interparietal, parietal, frontal and nasal bones), one fetus at 50 mg/kg bw per day (reduced interparietal bone), one fetus at 250 mg/kg bw per day (forelimb, absent ossification of the ulna; forepaw, adactyly) and one fetus at 500 mg/kg bw per day (absent ossification of the pubis) (Table 32). Skeletal anomalies consisted mainly of fused, fragmented or asymmetric sternebrae, irregular ossification of scapula, and displaced cervical and caudal vertebral centers. The incidence of these anomalies was not affected by treatment. The incidence of fused third and fourth sternebrae was slightly higher at 500 mg/kg bw per day than in the controls and was likely to be treatment-related (fetal incidence, 10.3%; litter incidence, 33.3%; range of incidences in historical controls, 0–5.4% and 0–29.4%, respectively). Skeletal variations occurred in about two-thirds of fetuses from almost all litters at all doses. They consisted mainly of poor or absent ossification of the first, fifth and sixth sternebrae, cranial findings (sutural bones, slot or hole in parietal bone), absent ossification of the first

Table 32. Fetal skeletal observations in a study of developmental toxicity in rabbits

	Dose (mg/kg bw per day)				
	0	10	50	250	500
Total fetuses examined/litters examined	116/19	130/18	90/16	97/17	97/18
Skeletal malformations (fetal incidence/litter incidence)					
Reduced interparietal bone	NF	1/1	1/1	NF	NF
Reduced parietal bone	NF	1/1	NF	NF	NF
Reduced frontal bone	NF	1/1	NF	NF	NF
Reduced nasal bone	NF	1/1	NF	NF	NF
Forelimb—absent ossification ulna	NF	NF	NF	1/1	NF
Fore paw—adactyly	NF	NF	NF	1/1	NF
Pelvic girdle—absent ossification pubis	NF	NF	NF	NF	1/1
Total skeletal malformations	NF	1/1	1/1	1/1	1/1
Treatment-related skeletal anomalies (fetal incidence/litter incidence)					
Asymmetrically shaped first sternebra	NF	1/1	1/1	2/1	3/1
Fused second and third sternebra	1/1	1/1	1/1	4/4	4/4
Asymmetrically shaped second sternebra	NF	1/1	1/1	2/2	4/3
Fused third and fourth sternebra	2/2	2/1	1/1	5/4	10*/6
Asymmetrically shaped third sternebra	NF	1/1	NF	2/2	3/3
Fused fourth and fifth sternebra	4/4	2/2	4/4	7/6	8/6
Asymmetrically shaped fourth sternebra	NF	1/1	NF	4/4	2/2
Total skeletal anomalies	12/8	9/7	7/6	21/12	21/
Total skeletal variations	98/19	107/18	74/16	80/16	77/17

From Khalil (1994b)
NF, not found
* $p < 0.05$; chi-squared test plus Fisher's Exact test

metacarpal, tail bone variations (poor or absent ossification of or additional caudalvertebral centres), additional ribs, and poor ossification of the medial phalanx of the fifth anterior digit. Poor ossification of the caudal vertebral centres was statistically significantly increased at 50 mg/kg bw per day (by 112.5% compared with controls). However, since there was no dose–response relationship, this result was not likely to be treatment-related. The incidence of fetuses with additional caudal vertebral centres was significantly lower at 10, 250 and 500 mg/kg bw per day when compared with controls, but this finding was not considered treatment-related by the consulting pathologist.

No teratogenic potential of trifloxystrobin was detected in rabbits. The NOAEL for maternal toxicity was 50 mg/kg bw per day on the basis of effects on body weight, food consumption, and food efficiency at the next higher dose. The NOAEL for developmental toxicity was 250 mg/kg bw per day on the basis of marginally increased incidences of skeletal anomalies of fused third and fourth sternebrae at the next higher dose (Khalil, 1994b).

2.6 Special studies

(a) Acute neurotoxicity

Rats

In a study of acute neurotoxicity conducted in compliance with the principles of GLP (with QA certification), groups of 10 male and 10 female Sprague-Dawley (Tif:RAIf) rats aged 5–7 weeks were given trifloxystrobin (purity, 96.4%; in 0.5% carboxymethylcellulose, 0.1% aqueous polysorbate 80) as a single oral dose at 2000 mg/kg bw by gavage. Animals in the control group received the vehicle alone. The design of this study was based on a previously conducted range-finding study in which no signs of toxicity were observed at doses of up to 2000 mg/kg bw. At 3500 mg/kg bw, reduced activity and piloerection were noted, being most prominent 6–8 h after treatment. Based on these data, a limit-test study using 2000 mg/kg bw, with a time of peak effect of 6 h, was considered adequate. The animals were checked twice per day for mortality and daily for clinical signs. Body weight was recorded at pre-test, day 1, and twice weekly, thereafter; food consumption was measured at pre-test and twice weekly, thereafter. FOB tests were conducted before the assessment of motor activity on randomized animals. Animals were observed in the home cage, during handling and in an open field. Tests of neurological function were performed at pre-test, day 1 (time of peak effect), day 8, and day 15 and included sensorimotor functions (approach, touch, vision, audition, pain, vestibular), autonomic functions (pupillary reflex, body temperature), and sensorimotor coordination (grip strength, landing foot splay). After conducting the FOB tests, motor activity was assessed using an automated open-field device to measure horizontal activity, vertical activity, and other parameters. At the end of the observation period, all animals were sacrificed by in-situ perfusion and submitted to macroscopic examination and tissue sampling of brain, spinal cord, and major peripheral nerves and ganglia. Histopathological examination of nervous system tissue was conducted on five animals of each sex per group.

A single male animal in the treatment group was found recumbent on day 2, had respiratory sounds and had to be sacrificed in a moribund condition. This incident was not considered to be related to treatment since the oral LD_{50} of the compound is known to be >5000 mg/kg bw. No other clinical signs or changes in behaviour were observed at any time during the study. Body-weight development and food consumption were not affected in treated animals.

Table 33. Motor activity in female rats at day 1 after receiving a single dose of trifloxystrobin

Parameter	Dose (mg/kg bw)		% decrease
	0 (control)	2000	
Total distance	2842 ± 805	1728 ± 890	39
Number of movements	193 ± 50	144 ± 72	36
Movement time	219 ± 50	136 ± 73	38
Vertical activity	554 ± 273	357 ± 200	35
Number of rearing	82 ± 37	57 ± 29	30
Vertical time	296 ± 159	188 ± 121*	36
Centre time	139 ± 95	34 ± 31*	75

From Classen (1997a)
* Significantly different from control at $p \leq 0.05$ (ANOVA)

The FOB tests revealed no neurological or behavioural effects of trifloxystrobin. Histopathological examination of tissues of the central and peripheral nervous system, the eyes, optic nerve and skeletal muscle did not show any treatment-related neuropathic changes. On day 1 (time of peak effect), female rats had statistically significantly decreased motor activity for both vertical and centre time (Table 33). There were no decreases in motor activity in males. The effect in females was considered to be treatment-related and likely to be caused by systemic toxicity rather than neurotoxicity.

The NOAEL was <2000 mg/kg bw on the basis of decreased motor activity in females (Classen, 1997a).

In a range-finding study of acute neurotoxicity conducted in compliance with the principles of GLP (with QA certification) to estimate the time of peak effect, groups of three male and three female Sprague-Dawley (Tif:RAIf) rats aged 5 weeks were given trifloxystrobin (purity 96.4%; in 0.5% carboxymethyl-cellulose, 0.1% aqueous polysorbate 80) as a single oral dose at 0, 1000, 2000 or 3500 mg/kg bw. Animals were observed for 4 days and measurements were recorded for body weight, food consumption, clinical signs, abbreviated FOB, and neurological assessment including sensorimotor function tests. Piloerection was seen in all animals at the highest dose at study day 1 and ended on study day 2. Reduced activity was observed at 2–4 h after administration of 2000 or 3500 mg/kg bw, reaching a maximum at 6–8 h after dosing. The reduced activity of males at the highest dose lasted for 3 days, while the remaining groups recovered by day 2. At 2000 mg/kg bw, low activity was noted only in two out of three males and not at all in the females. The effects, in general, were seen more clearly and started earlier and lasted longer in males than in females. On the basis of findings of this study, 2000 mg/kg bw was chosen as a limit dose for the actual study of acute oral neurotoxicity in rats (Classen, 1997b).

(b) Mechanistic studies

In the 3-month study of toxicity in rats (Gerspach, 1995), relative weights of the liver were increased in the males fed diets containing trifloxystrobin at a concentration of 500 or 2000 ppm with minimal hepatocyte hypertrophy at 2000 ppm (see section 2.2). The present study was conducted to assess possible induction of replicative DNA synthesis in the liver of male rats given diets containing trifloxystrobin for 3 months. For this purpose, formalin-fixed tissues from male rats in the same 3-month guideline feeding study were embedded and subjected to immunohistochemical analysis for proliferative cell nuclear antigen

(PCNA). Groups of five male rats were treated with trifloxystrobin admixed to the feed at dietary concentrations of 0, 100, 500 or 2000 ppm for 3 months, corresponding to target doses of 0, 6.44, 30.6 and 127 mg/kg bw per day. In order to test for the reversibilty of potential treatment-related changes, two additional groups of 10 animals received diets containing trifloxystrobin at 0 or 2000 ppm, corresponding to target doses of 0 and 127 mg/kg bw per day for 3 months followed by a recovery period of 28 days. Cells in S-phase of the cell cycle were identified by uniform nuclear staining for PCNA. Hepatocytes but not sinusoidal cells were evaluated for PCNA-positive nuclei using a microscope connected to a Vidas image analysis system. Cells and PCNA-positive nuclei were counted in 10 microscopic fields per animal, giving a total area of 4.44 mm^2. A total of approximately 800 cells were counted per animal.

For each animal, a labelling index for hepatocytes was calculated as follows:

$$\text{Labelling index (\%)} = 100 \times \frac{\text{PCNA positive nuclei} / \text{mm}^2 \text{ investigated area}}{\text{Total number of nuclei} / \text{mm}^2 \text{ investigated area}}$$

Treatment with trifloxystrobin at all doses investigated did not increase the mean hepatocyte nuclear labelling indices (Table 34).

In conclusion, there was no evidence for induction of replicative DNA synthesis in hepatocytes of male rats after 3 months of treatment with trifloxystrobin. However this study is of limited value as proliferation may only occur within the first few days of exposure (Persohn, 1995a).

PCNA-dependent eplicative DNA synthesis was also investigated in livers of male mice from a 3-month range-finding dietary study of toxicity (Gerspach, 1994a). In that study, males and females in groups receiving trifloxystrobin at dietary concentrations of 2000 or 7000 ppm had increased absolute and relative weights of the liver; in addition, there were increased incidences of centrilobular hepatocyte hypertrophy at 7000 ppm and hepatocyte necrotic changes at 2000 and 7000 ppm (see above section 2.2; Gerspach, 1994a). The present study was conducted to assess possible induction of replicative DNA synthesis in livers of male mice after dietary administration of trifloxystrobin for 3 months. Liver samples from groups of 10 male mice fed diets containing trifloxystrobin at a concentration of 0, 500, 2000 or 7000 ppm, corresponding to target doses of 0, 76.9, 315 and 1275 mg/kg bw per day, respectively, were embedded and subjected to immunohistochemical analysis for PCNA, as described above. The same procedure as used in the study in rats

Table 34. Labelling index as measured by PCNA-positive nuclei in livers of male rats fed diets containing trifloxystrobin for 3 months

Dietary concentration (ppm)	Mean labelling index index (% PCNA-positive nuclei)	
	After termination of treatment	After 4 weeks of recovery[a]
0	1.58 ± 0.51	1.05 ± 0.55
100	0.92 ± 0.24	ND
500	0.85 ± 0.33	ND
2000	1.22 ± 0.17	1.24 ± 0.29

From Persohn (1995a)
ND, not determined
[a] Mean of 5 or 10 animals each ± standard deviations

was also used to identify and count uniformly-stained PCNA-positive nuclei, except that a total of 600 S-phase cells per animal were counted, rather than 800. The labelling index was also counted in the same manner.

Immunohistochemical staining of sections of male mouse liver for PCNA did not reveal any increase in the fraction of DNA-synthesizing hepatocytes in S-phase in cells from mice at all doses investigated (Table 35).

In conclusion, there was no evidence for induction of replicative DNA synthesis in hepatocytes of male mice after 3 months of treatment with trifloxystrobin. However, this study is of limited value as proliferation may only occur within the first few days of exposure (Persohn, 1995b).

Trifloxystrobin and its carboxylic acid metabolite, CGA 321113, were tested for cytotoxicity in cultures of hepatocytes, and for inhibition of mitochondrial function.

Hepatocytes from a young adult male Wistar rat (Crl(WI)BR) were isolated by liver perfusion with collagenase and, using standard procedures, were cultured in 24-well plates. To the hepatocyte cultures were added (for 1–24 h) different concentrations of trifloxystrobin (10, 30, 60, 100, 300 and 600 nmol/l) and its carboxylic acid metabolite, CGA 321113, (1000, 10 000, 30 000 nmol/l). Both test chemicals were added in DMSO at a final concentration of 0.1% DMSO in culture. After 1, 4 and 24 h of treatment, the following morphological changes were recorded and graded (1–3): irregular cell surface, formation of blebs, cell spreading, intracellular granulation or vacuolization and cell disaggregation. Cell death and/or complete detachment of the monolayer were also recorded. At 4 h and 24 h after initiation of treatment, lactate dehydrogenase (LDH) activity was determined in the culture medium spectrophotometrically. Total intracellular LDH activity was determined from three additional cultures that were sonicated just before starting treatment. LDH release was expressed as a percentage of total intracellular activity.

Freshly prepared liver mitochondria from an overnight fasted young adult male Tif: RAlf (SPF) rat were used for analysis of oxygen consumption using a biological oxygen monitor connected to a recorder and a polarographic oxygen probe, calibrated at 100% with air-saturated water. Reaction chambers were maintained at 30 °C. Five seconds after addition of a sample of mitochondrial fraction to a Tris-potassium phosphate buffer (pH 7.4) reaction medium, succinate (10 mmol/l) was added as site II metabolic substrate in the presence of rotenone (30 µmol/l), an inhibitor of site I. Subsequent addition of ADP (300 nmol/l) generated state 3 (ADP-stimulated) respiration. After returning to state 4 respiration (resting respiration), the test article dissolved in DMSO was added (0.1% final concentration of DMSO) and state 3 and 4 respiration were again measured.

Table 35. Labelling index as measured by PCNA-positive nuclei in livers of male mice fed diets containing trifloxystrobin for 3 months

Dietary concentration (ppm)	Mean labelling index (% PCNA-positive nuclei)[a]
0	0.68 ± 0.26
500	1.11 ± 0.65
2000	0.66 ± 0.36
7000	0.71 ± 0.38

From Persohn (1995b)

[a] Mean of 10 animals each ± standard deviations

Incubation of cultured rat hepatocytes with trifloxystrobin at a concentration range of 5–100 µmol/l resulted in a rapid and marked degenerative change of the cell structure at 30 and 100 µmol/l with cell death occurring after 4 h and 24 h after treatment. CGA 321113 was much less toxic and caused no morphological changes at a concentration of less than 600 µmol/l. Accordingly, significant LDH leakage was measured after treatment with trifloxystrobin at concentrations of 30 µmol/l and greater, while treatment with CGA 321113 caused an increased LDH leakage at 600 µmol/l only. CGA 321113 was shown to be 20 times less cytotoxic than trifloxystrobin in cultured rat hepatocytes.

Rates of mitochondrial respiration were assessed before and after the addition of the trifloxystrobin or CGA 321113 in the presence of succinate as a substrate. The respiratory control ratio, RCR (state 3 respiration/state 4 respiration) provides a measure of mitochondrial integrity and is an indicator of the "tightness of coupling" in mitochondria. The ADP : O ratio (or P : O ratio), which is equal to moles of ADP phosphorylated per mole of atomic oxygen consumed, is calculated as an index of oxidative phosphorylation. Trifloxystrobin inhibited state 3 and state 4 mitochondrial respiration in a concentration-dependent manner, with IC_{50} values of 68 and 154 nmol/l, respectively. Concentration-dependent decreases of the RCR and P : 0 were observed with trifloxystrobin at concentrations of between 10 and 100 nmol/l. In contrast, CGA 321113 did not inhibit mitochondrial respiration at concentrations of up to 30 000 nmol/l.

In conclusion, trifloxystrobin was cytotoxic in cultures of rat hepatocytes and inhibited mitochondrial respiration and oxidative phosphorylation in the mammalian liver; CGA 321113, a major metabolite found in rats and goats, was far less cytotoxic and did not inhibit mitochondrial function at concentrations of up to three orders of magnitude higher than trifloxystrobin (Bouis, 1997).

(c) Studies with metabolites

(i) Acute oral toxicity

Studies of acute oral toxicity (limit test) in rats were conducted using CGA 357261 (the *Z*,*E*-isomer of trifloxystrobin) or one of four metabolites, namely CGA 373466, NOA 414412, NOA 413161, or NOA 413163. A single oral dose of 2000 mg/kg bw was administered to five rats of each sex, and the animals were observed for 14 days. All animals survived to the scheduled sacrifice. A summary of the results is presented in Table 36.

(ii) Genotoxicity

Trifloxystrobin's metabolites CGA 373466 and NOA 414412, NOA 413161, and NOA 413163, in addition to CGA 357261 (*Z*,*E*-isomer of trifloxystrobin), were tested in assays for reverse gene mutation in bacteria in the presence or absence of a metabolic activation

Table 36. Acute oral toxicity of metabolites of trifloxystrobin in male and female rats

Metabolite	Strain	LD50 (mg/kg bw)	Reference
CGA 357261	Sprague Dawley (Tif : RAI)	>2000	Winkler (1997)
CGA 373466	Wistar Han	>2000	Cantoreggi (1997a)
NOA 414412	Wistar Han	>2000	Cantoreggi (1997b)
NOA 413161	Wistar Han	>2000	Cantoreggi (1998a)
NOA 413163	Wistar Han	>2000	Cantoreggi (1998b)

Table 37. Results of assays for reverse mutation in vitro with metabolites of trifloxystrobin

Metabolite	Test object[a]	Concentration (solvent)	Result	Reference
CGA 357261	*S. typhimurium*; *E. coli*	312.5–5000 µg/plate, ±S9 (DMSO)	Negative	Deparade (1997a)
CGA 373466	*S. typhimurium; E. coli*	20.6–5000 µg/plate, ±S9 (DMSO)	Negative	Deparade (1997b)
NOA 414412	*S. typhimurium; E. coli*	312.5–5000 µg/plate, ±S9 (DMSO)	Negative	Deparade (1997c)
NOA 413161	*S. typhimurium; E. coli*	312.5–5000 µg/plate, ±S9 (DMSO)	Negative	Deparade (1998a)
NOA 413163	*S. typhimurium; E. coli*	312.5–5000 µg/plate, ±S9 (DMSO)	Negative	Deparade (1998b)

[a] The same strains were used in all tests: *S. typhimurium* TA 98, TA 100, TA 102, TA 1535 and TA 1537, and *Escherichia coli* WP2uvrA

system (S9). All results were negative up to the limit tested dose of 5000 µg/plate (Table 37).

3. Observations in humans

At present there are very few data on human exposure to trifloxystrobin, thus no firm conclusions can be drawn. In a recent update by the sponsor, there are no new data on human exposure.

3.1 Literature search

According to a recent open search of various international databases of medical literature conducted by Bayer, no reports of poisoning cases have been recorded (Heimann, 2004).

3.2 Occupational health surveillance

Manufacturing employees in Switzerland are medically examined by a company physician at the beginning of their employment and then routinely once per year according to the criteria of the Swiss Accident Insurance Institution (SUVA).

Routine medical examinations include: anamnesis; physical examination including blood pressure; blood analysis (including haemoglobin, erythrocytes, leukocytes, thrombocytes, leukocyte differentiation, blood sedimentation rate, blood sugar, cholesterol, triglycerides, alanine aminotransferase, aspartate amino transferase, alkaline phosphatase, bilirubin, creatinine, urea, uric acid); and urine analysis.

Trifloxystrobin has been formulated in a pilot plant (EZA) at Münchwilen (Switzerland) since 1996. Manufacturing is performed in campaigns with a total of about 10 campaigns per year. The average duration of a campaign is 1–2 days. The annual rate of production of different formulations is in the range of 1–100 kg per formulation. Four to five formulations had a production volume of 500 kg. The total formulation volume was 2.5 tonnes/year.

Annually, a total of 10 workers are involved in the formulation campaigns of trifloxystrobin. Questionnaires filled in by the head of the manufacturing site and by the responsible occupational physician revealed that no adverse health effect which could be related to trifloxystrobin was observed during this period.

A recent update by Bayer indicated that, during the production period from August 1, 2000 to May 17, 2004, there were no accidents or undesirable symptoms (based on the

above listed laboratory and medical tests) among 34 plant employees (Fehling Voigt & Gatz, 2004).

3.3 Cases reported to the company (European Union dossier)

During field-trial applications in South Africa in March 1996, one case of skin and eye irritation was reported. The symptoms occurred while weighing the product WG-type formulation) into plastic bags. The person experienced a burning sensation in the eyelids and nose tissue and also slightly in the chest; these symptoms started within 5 min after beginning of work and lasted up to 30–45 min after termination of weighing. Washing of hands and face several times during work did not reduce the severity or duration of the symptoms. The effects occurred for the first time during weighing the product for the second application and thereafter every time when weighing the product. No symptoms were noted during spraying in the field (using a knapsack). Protective clothing consisted of overalls but no gloves and no eye/face protection. The person had never experienced similar symptoms before.

In June 1996, two other cases were reported from Germany. Two persons started working in vineyards at two different locations 1–2 h after application of different products, including WG-type formulations of trifloxystrobin. Both workers went home after work with considerable irritation of the eyes and the skin (in one case). Further details of these two cases are not known. Which of the applied products might have been responsible for the observed effects had not been carefully evaluated. There is insufficient evidence for a major contribution of trifloxystrobin to the reported effects.

To learn more about the irritating potential of formulations containing trifloxystrobin, a questionnaire was distributed at the end of July 1996 to all locations worldwide where field trials with these products were ongoing. A total of 13 countries were contacted, and replies were obtained between August and October 1996 from 11 of these. More than 120 people were involved in field trials in these 11 countries. None of these people had ever experienced any irritating effects during their work with formulations of trifloxystrobin.

In conclusion, based on experience of more than 120 persons involved in field trials in 11 countries all over the world with different formulations of trifloxystrobin, these products were considered to have no intrinsic irritation potential to humans. This is in agreement with data from testing in animals. The significane of two reported cases from Germany is inconclusive because of the broad spectrum of products applied in these trials. For the case reported from South Africa, an allergic reaction of this particular individual to the dry product cannot be excluded. Testing in animals has revealed that the active ingredient has sensitizing potential.

Comments

After oral administration, radiolabelled trifloxystrobin was rapidly and appreciably absorbed (66% of the administered dose) in rats of both sexes. The major route of elimination (63–84%) was in the faeces; some of the faecal elimination was via bile (30–45%) while only one-third or less of the administered dose was excreted in the urine, and none through expired air. There was almost complete degradation of trifloxystrobin after single low dose at 0.5 mg/kg bw, but up to 45% was eliminated unchanged in the faeces after administration of the highest dose at 100 mg/kg bw. The pattern of metabolites in rats is very

complex; about 35 metabolites were identified in the urine, faeces, and bile. The major steps in the metabolic pathway include hydrolysis of the methyl ester to the corresponding acid, *O*-demethylation of the methoxyimino group yielding a hydroxyimino compound, and oxidation of the ethylideneamino methyl group to a primary alcohol, and then to the corresponding carboxylic acid. These steps are followed by a complex pattern of further, minor reactions. Cleavage between the glyoxylphenyl and trifluormethylphenyl moieties accounted for about 10% of the administered dose.

The metabolism of trifloxystrobin in plants is similar to that in animals, and occurs primarily via cleavage of the methyl ester group to form CGA 321113 (*E*,*E*)-methoxyimino-{2-[1-(3-trifluoro methyl-phenyl)-ethylideneaminooxymethyl]-phenyl}-acetic acid. In the rat, this metabolite undergoes further hydroxylation and conjugation (glucuronide and sulfate) at the trifluoromethyl phenyl ring. In goat liver, taurine and glycine conjugates of CGA 321113 were the principal residue components (up to 28% of the total radioactive residues). Conjugated metabolites are generally less toxic and more rapidly excreted than the unconjugated parent compound. Being biotransformation products in the rat, CGA 321113 and its metabolites are assumed to have been adequately tested and accounted for in rats given trifloxystrobin. Also, CGA 321113 is not likely to be more toxic than trifloxystrobin.

Dermal absorption of trifloxystrobin in rats was low and decreased slightly with increasing dose. In a test in vitro, rat epidermis was nine and 19 times more permeable to trifloxystrobin at a dose of 0.24 and 10.27 mg/cm^2, respectively, than was human epidermis. In a study of absorption in vivo in which a low or a high dose of radiolabelled trifloxystrobin was applied to the shaved backs of male rats, the amount of recovered radioactivity in the blood was low, but the overall absorption was moderate, ranging from 5% to 10% in 24 h and increasing to 16% at 48 h.

Trifloxystrobin has low acute oral toxicity in rats and mice (LD_{50} > 5000 mg/kg), low acute dermal toxicity in rats and rabbits (LD_{50} > 2000 mg/kg), low acute inhalation toxicity in rats (LC_{50} > 4.65 mg/l), is not a skin irritant in rabbits, is a moderate eye irritant in unwashed rabbit eyes but is not irritating in washed rabbit eyes. It is a skin sensitizer in guinea-pigs, according to the Magnusson & Kligman maximization test, but is not a skin sensitizer in guinea-pigs according to the Buehler test.

In studies of toxicity with repeated doses, slight decreases (5–10%) in body weight and/or body-weight gain were regarded as non-adverse in the absence of other effects.

In studies of repeated doses in mice, the liver and spleen were the principal target organs at the same or higher doses than those affecting body weight and food efficiency. In the 90-day study in male and female mice, liver weight was increased and there were findings on microscopy, including hepatocyte hypertrophy and focal or single cell necrosis. There were also increased incidences of extramedullary haematopoiesis in the spleen at doses of ≥315 mg/kg bw per day. The NOAEL for these effects was 77 mg/kg bw per day.

In a 90-day dietary study in rats, the NOAEL was 31 mg/kg bw per day on the basis of statistically significantly decreased body-weight gain of 20% and 40% in males and females, respectively, increased relative liver weights, changes in clinical chemistry, and liver histopathology findings (mainly hepatocellular hypertrophy), in addition to atrophy of the pancreas at the next higher dose of 127 mg/kg bw per day.

At or above a daily dose of trifloxystrobin at 150 mg/kg bw per day for 3 months or 50 mg/kg bw per day for 1 year, dogs had episodes of diarrhoea, vomiting, reduced food intake, increased relative weight of the liver, and hepatocyte hypertrophy, in addition to changes in clinical chemistry parameters indicative of liver toxicity and/or perturbed metabolism, dehydration, poor nutrition, and possible starvation. Body weights were also affected. In the 3-month study, animals of both sexes had body-weight loss of about 0.4 kg and 2.8 kg at 150 and 500 mg/kg bw per day, respectively. In the 1-year study, body-weight gain in females at 50 and 200 mg/kg bw per day was decreased throughout the study, and at week 52 body-weight gain was about 20% below control values. The NOAELs were 30 and 5 mg/kg bw per day in the 3-month and 1-year studies, respectively.

Long-term study of toxicity and carcinogenicity with trifloxystrobin were carried out in mice and rats. In the 18-month dietary feeding study in mice, the NOAEL was 36 mg/kg bw per day on the basis of liver effects, including increased weight of the liver (both sexes) and increased single-cell necrosis (males), in addition to impaired body-weight gain (females). There was no evidence of carcinogenicity in mice tested at adequate doses.

In the 2-year study in rats, the NOAEL was 30 mg/kg bw per day on the basis of statistically significantly retarded body-weight gain in males (11–17%) and females (17–27%) and decreased food consumption (by 4% and 8%, respectively) and increased relative weights of heart, liver, and kidneys (each by about 20%) in females at the highest dose of 62 mg/kg bw per day. The overall incidence of tumours was lower in the treated animals. Benign adrenal medullary tumours (10% versus 0% in controls) and haemangioma in the mesenteric lymph nodes (10.2% versus 0% in controls) were increased in male rats at the highest dose tested. Incidences of the adrenal medullary tumours were within the range of incidences for historical controls. The incidence of haemangioma in the mesenteric lymph nodes in males at the highest dose group was outside the range of incidences for historical controls. There was markedly reduced mortality in the group receiving the highest dose tested, and this may have contributed to the higher incidence of tumours in this group compared with controls. In ageing male rats of this strain, degenerative lesions associated with the mesenteric lymph nodes are common and are hard to distinguish from neoplastic lesions (haemangiomas). Some age-associated non-neoplastic findings, such as angiomatous hyperplasia of the mesenteric lymph nodes, were increased in males at the highest dose and the increases were correlated with decreased food intake and a lower body-weight development.

The Meeting concluded that trifloxystrobin had no treatment-related carcinogenicity of any toxicological concern.

A wide range of assays for genotoxic potential with trifloxystrobin were conducted in vitro and in vivo, including testing for gene mutation, chromosomal damage and DNA repair. Trifloxystrobin was weakly mutagenic at cytotoxic doses in the test for forward gene mutation in Chinese hamster V79 cells. Results were equivocal in the absence of metabolic activation. Metabolites of trifloxystrobin [CGA 357261 (*Z*, *E*-isomer), CGA 373466, and NOA 414412] were not mutagenic in the Ames test. The Meeting concluded that trifloxystrobin and its metabolites are not genotoxic.

Because of the absence of findings indicative of genotoxicity or carcinogenicity, the Meeting concluded that trifloxystrobin is unlikely to pose a carcinogenic risk to humans.

In the two-generation study in rats given trifloxystrobin at a dose of 55 or 111 mg/kg bw per day, pups in the F_1 and F_2 litters had retarded body-weight development during lactation. The NOAEL for parental toxicity was 3.8 mg/kg bw per day on the basis of findings at 55 mg/kg bw per day, i.e. reduced body weight and food consumption, in addition to histopathology findings in the liver and kidneys. The NOAEL for offspring toxicity was 3.8 mg/kg bw per day on the basis of retarded body-weight development during lactation. The NOAEL for reproductive toxicity was 111 mg/kg bw per day.

Trifloxystrobin was not teratogenic in rats and rabbits when tested at doses of up to 1000 and 500 mg/kg bw per day, respectively. In rats, the NOAEL for developmental toxicity was 100 mg/kg bw per day on the basis of increased incidences of enlarged thymus. In rabbits, the NOAEL for developmental toxicity was 250 mg/kg bw per day on the basis of increased incidences of skeletal anomalies in the form of fused sternebrae 3 and 4. Maternal toxicity in rats and rabbits was limited to reduced food consumption and body-weight loss at 100 and 250 mg/kg bw per day with NOAELs of 10 and 50 mg/kg bw per day, respectively. The developmental effects were considered to be a consequence of overall maternal toxicity.

The Meeting concluded that the existing database on trifloxystrobin was adequate to characterize the potential hazards to fetuses, infants, and children.

In a study of acute oral neurotoxicity in rats given a single dose of trifloxystrobin at 2000 mg/kg bw, the functional observational battery revealed no indications for potential neurological or behavioural effects.

Toxicological evaluation

The Meeting established an ADI of 0–0.04 mg/kg bw based on the parental NOAEL of 3.8 mg/kg bw per day in a multigeneration study of reproductive toxicity in rats and a 100-fold safety factor. The lowest-observed-adverse-effect level (LOAEL) was 55 mg/kg bw per day on the basis of effects on body weight and food consumption, in addition to liver and kidney histopathology findings. This value is supported by the NOAEL of 5 mg/kg bw per day in the 1-year study in dogs.

The Meeting concluded that it was unnecessary to establish an ARfD for trifloxystrobin on the basis of its low acute toxicity and the fact that developmental effects were considered to be a result of severe maternal toxicity, which is related to decreased food intake rather than systemic toxicity. Also, the vomiting and diarrhoea observed in dogs were clearly related to local irritation, rather than systemic acute toxicity.

Levels relevant to risk assessment

Species	Study	Effect	NOAEL	LOAEL
Mouse	18-Month study of toxicity and carcinogenicitya	Toxicity	300 mg/kg, equal to 36 mg/kg bw per day	1000 mg/kg, equal to 124 mg/kg bw per day
		Carcinogenicity	2000 mg/kg, equal to 246 mg/kg bw per dayb	—
Rat	2-Year studies of toxicity and carcinogenicity[a]	Toxicity	750 mg/kg, equal to 30 mg/kg bw per day	1500 mg/kg, equal to 62 mg/kg bw per dayb
		Carcinogenicity	1500 mg/kg, equal to 62 mg/kg bw per dayb	—
	Two-generation reproductive toxicity[a]	Parental toxicity	50 mg/kg, equal to 3.8 mg/kg bw per day	750 mg/kg, equal to 55 mg/kg bw per day
		Offspring toxicity	50 mg/kg, equal to 3.8 mg/kg bw per day	750 mg/kg, equal to 55 mg/kg bw per day
	Developmental toxicity[c]	Maternal toxicity	10 mg/kg bw per day	100 mg/kg bw per day
		Embryo- and fetotoxicity	100 mg/kg bw per day	1000 mg/kg bw per day
Rabbit	Developmental toxicity[c]	Maternal toxicity	50 mg/kg bw per day	250 mg/kg bw per day
		Embryo- and fetotoxicity	250 mg/kg bw per day	500 mg/kg bw per day
Dog	3-Month study of toxicity[d,e]	Toxicity	30 mg/kg bw per day	150 mg/kg bw per day
	12-Month study of toxicity[d]	Toxicity	5 mg/kg bw per day	50 mg/kg bw per day

[a] Diet
[b] Highest dose tested
[c] Gavage
[d] Gelatin capsule
[e] Two or more studies combined

Estimate of acceptable daily intake for humans

0–0.04 mg/kg bw

Estimate of acute reference dose

Unnecessary

Studies that would provide information useful for continued evaluation of the compound

Further observations in humans

Summary of critical end-points for trifloxystrobin

Absorption, distribution, excretion and metabolism in animals	
Rate and extent of absorption	66% in 48 h
Distribution	Widely distributed; highest concentrations in blood, liver, and kidneys
Potential for accumulation	No potential for accumulation.
Rate and extent of excretion	Within 48 h, 72–96% of the administered dose is eliminated in the urine and faeces
Metabolism in animals	Extensive: hydrolysis, *O*-demethylation, oxidation, conjugation, chain shortening, and cleavage between glyoxylphenyl and trifluoromethyl moieties
Toxicologically significant compounds (plants, animals and the environment)	Parent compound, major acid metabolite is CGA 321113
Acute toxicity	
Rat, LD_{50}, oral	>5000 mg/kg bw
Rat, LD_{50}, dermal	>2000 mg/kg bw
Rat, LC_{50}, inhalation:	>4.6 mg/l air
Rabbit, skin irritation:	Not irritating
Rabbit, eye irritation:	Not irritating
Skin sensitization	Sensitizer (Magnusson & Kligman test)
Short-term studies of toxicity	
Target/critical effect	Body weight, food consumption, clinical signs, liver (pathology), kidney (weight), pancreas (atrophy), spleen (weight and pathology)
Lowest relevant oral NOAEL	5 mg/kg bw per day (1-year study in dogs)
Lowest relevant dermal NOAEL	≥1000 mg/kg bw per day (28-day study in rats)
Lowest relevant inhalation NOAEC	No relevant study
Genotoxicity	No genotoxic potential, negative results in vivo, one positive result in study in vitro at cytotoxic doses.
Long-term studies of toxicity and carcinogenicity	
Target/critical effect	Body weight (mouse, rat), food consumption (rat), liver (mouse, rat)
Lowest relevant NOAEL	30 mg/kg bw per day (2-year study in rats)
Carcinogenicity	Unlikely to pose a carcinogenic risk to humans
Reproductive toxicity	
Target/critical effect	Decreased body-weight gain of pups accompanied by delayed eye opening at parental toxic doses
Lowest relevant reproductive NOAEL	50 ppm (3.8 mg/kg bw per day)s
Developmental target/critical effect	Enlarged thymus (rat) and skeletal effects (rabbit) at maternally toxic doses
Lowest relevant developmental NOAEL	100 mg/kg bw per day (rat)
Neurotoxicity	No evidence of acute neurotoxicity in rats
Other toxicological studies	No evidence of replicative DNA synthesis in rat or mouse heptocytes after 3-months administration in diet A range of metabolites had low acute oral toxicity and there was no evidence of genotoxic activity
Medical data	New active substance; limited data; some evidence of skin and eye irritation in three people during field trials (but 120 people without effects)

Summary

	Value	*Study*	*Safety factor*
ADI	0–0.04 mg/kg bw	Rat, reproduction study, reduced body weight, liver and kidney effects	100
ARfD	Unnecessary	—	—

References

Altmann, B. (1994) 28-Day range finding toxicity study in beagle dogs. Unpublished Bayer AG report No. 933163, dated 28 September 1994, from Ciba-Geigy Ltd, Stein, Switzerland.

Altmann, B. (1996) 3-Month subchronic oral toxicity study in beagle dogs. Unpublished Bayer AG report No. 943040, dated 26 June 1996 from Ciba-Geigy Ltd, Stein, Switzerland.

Altmann, B. (1997) 12-Month chronic oral toxicity study in beagle dogs. Unpublished Bayer AG report No. 943041, dated 2 December 1997 from Novartis Crop Protection AG, Stein, Switzerland.

Bouis, P. (1997) Cytotoxicity in primary cultured rat hepatocytes and effects on mitochondrial function of rat liver. Unpublished Bayer AG report No. CB 97/59, dated 17 December 1997, from Novartis crop Protection AG, Basel, Switzerland.

Buehler, E.V. Delayed contact hypersensitivity in the guinea-pig. *Arch. Dermatol*., **91**, 171–175.

Cantoreggi, S. (1997a) Acute oral toxicity in the rat (limit test). Unpublished Bayer AG report No. 973024, dated 18 July 1997, from Novartis Crop Protection AG, Stein, Switzerland.

Cantoreggi, S. (1997b) NOA 414412 tech. (metabolite of trifloxystrobin)—acute oral toxicity in the rat (limit test). Unpublished Bayer AG report No. 973064, dated 20 October 1997, from Novartis Crop Protection AG, Stein, Switzerland.

Cantoreggi, S. (1998a) NOA 413161 tech. (metabolite of trifloxystrobin)—acute oral toxicity in the rat (limit test). Unpublished Bayer AG report No. 983068, dated 18 September 1998, from Novartis Crop Protection AG, Stein, Switzerland.

Cantoreggi, S. (1998b) NOA 413163 tech. (metabolite of trifloxystrobin)—acute oral toxicity in the rat (limit test). Unpublished Bayer AG report No. 983103, dated 18 August 1998, from Novartis Crop Protection AG, Stein, Switzerland.

Classen, W. (1997a) Acute oral neurotoxicity study in rats. Unpublished Bayer AG report No. 973005, dated 2 December 1997, from Novartis Crop Protection AG, Stein, Switzerland.

Classen, W. (1997b) Acute oral rangefinding neurotoxicity study in rats. Unpublished Bayer AG report No. 791–97, dated 10 September 1997, from Novartis crop Protection Inc., Greensboro NC, USA.

De Bie, A. (1997) Dermal absorption study with [glyoxyl-phenyl-U-^{14}C] trifloxystrobin formulated as A-9604 A in rats. Unpublished Bayer AG report No. 470955, dated 10 December 1997, from Novartis Crop Protection AG, Basel, Switzerland.

Deparade, E. (1997a) *Salmonella* and *Escherichia*/mammalian-microsome mutagenicity test. Unpublished Bayer AG report No. 973007, dated 18 March 1997, from Novartis crop Protection AG, Stein, Switzerland.

Deparade, E. (1997b) *Salmonella* and *Escherichia*/mammalian-microsome mutagenicity mest. Unpublished Bayer AG report No. 973025, dated 16 September 1997, from Novartis crop Protection AG, Stein, Switzerland.

Deparade, E. (1997c) NOA 414412 tech. (metabolite of trifloxystrobin)—*Salmonella* and *Escherichia*/mammalian-microsome mutagenicity test. Unpublished Bayer AG report No. 973065, dated 29 October 1997, from Novartis crop Protection AG, Stein, Switzerland.

Deparade, E. (1998a) NOA 413161 tech. (metabolite of trifloxystrobin)—*Salmonella* and *Escherichia*/mammalian-microsome mutagenicity test. Unpublished Bayer AG report No. 973069, dated 16 September 1998, from Novartis crop Protection AG, Stein, Switzerland.

Deparade, E. (1998b) NOA 413163 tech. (metabolite of trifloxystrobin)—*Salmonella* and *Escherichia*/mammalian-microsome mutagenicity test. Unpublished Bayer AG report No. 983104, dated 29 September 1998, from Novartis crop Protection AG, Stein, Switzerland.

Draize, J.H. (1959) Primary irritation of the skin. In: Association of Food and Drug Officials of the United States, *Appraisal of the safety of chemicals in foods*, *drugs and cosmetics—dermal toxicity*, pp. 46–47.

Draize, J.H. (1959) Eye mucosa. In: Association of Food and Drug Officials of the United States, *Appraisal of the safety of chemicals in foods*, *drugs and cosmetics—dermal toxicity*, pp. 49–50.

Fehling Voit, U. & Gatz, U. (2004) Occupational medical experiences with trifloxystrobin technical. Unpublished report dated 18 May 2004 from Bayer (Schweiz) AG.

Fitzgerald (1993) Trifloxystrobin technical, range-finding rat oral teratogenicity. Unpublished report No. 943340, dated 9 June 1993, from Novartis Crop Protection AG, Stein, Switzerland.

Gerspach, R. (1994a) 3-Months range finding toxicity study in mice (administration in food). Unpublished Bayer AG report No. 933165, dated 14 November 1994, from Ciba-Geigy Ltd, Stein, Switzerland.

Gerspach, R. (1994b) 28 Days range finding study in rats (administration in food). Unpublished Bayer AG report No. 933099, dated 4 February 1994, from Ciba-Geigy Ltd, Stein, Switzerland.

Gerspach, R. (1995) 3-Months oral toxicity study in rats (administration in food). Unpublished Bayer AG report No. 933164, dated 19 January 1995, from Ciba-Geigy Ltd, Stein, Switzerland.

Gerspach, R. (1996) 28-Day repeated dose dermal toxicity study in the rat. Unpublished Bayer AG report No. 943046, dated 5 March 1996, from Ciba-Geigy Ltd, Stein, Switzerland.

Gerspach, R. (1997a) 18-Months carcinogenicity study in mice. Unpublished Bayer AG report No. 943039, 22 October 1997, from Novartis Crop Protection AG, Stein, Switzerland.

Gerspach, R. (1997b) 24-Month carcinogenicity and chronic toxicity study in rats. Unpublished Bayer AG report No. 943038, dated 22 October 1997, from Novartis Crop Protection AG, Stein, Switzerland.

Glaza, S.M. (1994a) Acute oral toxicity study of trifloxystrobin technical in rats. Unpublished Bayer AG report No. HWI 40702444, dated 5 October 1994, from Hazelton Wisconsin Inc., Madison, Wisconsin, USA.

Glaza, S.M. (1994b) Acute dermal toxicity study of trifloxystrobin technical in rabbits. Unpublished Bayer AG report No. HWI 40702445, dated 7 October 1994, from Hazelton Wisconsin Inc., Madison, Wisconsin, USA.

Glaza, S.M. (1994c) Primary dermal irritation study of trifloxystrobin technical in rabbit. Unpublished Bayer AG report No. HWI 40702446, dated 5 October 1994, from Hazelton Wisconsin Inc., Madison, Wisconsin, USA.

Glaza, S.M. (1994d) Primary eye irritation study of trifloxystrobin technical in rabbit. Unpublished Bayer AG report No. HWI 40702447, dated 7 October 1994, from Hazelton Wisconsin Inc., Madison Wisconsin, USA.

Glaza, S.M. (1994e) Dermal sensitisation study of trifloxystrobin technical in guinea pigs—closed patch technique. Unpublished Bayer AG report No. HWI 40702448, dated 18 November 1994, from Hazelton Wisconsin Inc., Madison Wisconsin, USA.

Heimann, K.G. (2004) Trifloxystrobin: assessment of literature research in various data bases. Unpublished Bayer CropScience AG report dated 6 May 2004.

Hertner, T. (1994a) *Salmonella* and *Escherichia*/mammalian-microsome mutagenicity test. Unpublished Bayer AG report No. 943074, dated 26 September 1994, from Ciba-Geigy Ltd, Basel, Switzerland.

Hertner, T. (1994b) Cytogenetic test on Chinese hamster cells in vitro (EC-Conform). Unpublished Bayer AG report No. 943076, dated 6 December 1994, from Ciba-Geigy Ltd, Basel, Switzerland.

Hertner, T. (1995a) Gene mutation test with Chinese hamster cells V79. Unpublished Bayer AG report No. 943075, dated 5 July 1995, from Ciba-Geigy Ltd, Basel, Switzerland.

Hertner, T. (1995b) Autoradiographic DNA repair test on rat hepatocytes in vitro. Unpublished Bayer AG report No. 943077, dated 9 June 1995, from Ciba-Geigy Ltd, Basel, Switzerland.

Hertner, T. (1995c) Micronucleus test mouse. Unpublished Bayer AG report No. 943078, dated 1 February 1995, Ciba-Geigy Ltd, Basel, Switzerland.

Holbert, M.S. (1995) Acute inhalation toxicity study in the rat. Unpublished Bayer AG report No. 1815–95, dated 5 April 1995, from Stillmeadow Inc., Houston, Texas, USA.

Khalil, S. (1994a) Trifloxystrobin technical, range-finding rabbit oral teratogenicity. Unpublished report Novartis Crop Protection Test No. 933142, Novartis Nexus Number 498-94, dated 18 March 1994.

Khalil, S. (1994b) Rabbit oral teratogenicity. Unpublished Bayer AG report No. 943043, dated 21 December 1994, from Ciba-Geigy Ltd, Stein, Switzerland.

Khalil, S. (1995) Rat oral teratogenicity. Unpublished Bayer AG report No. 943042, dated 7 March 1995, from Ciba-Geigy Ltd, Stein, Switzerland.

Khalil, S. (1997) Rat dietary two-generation reproduction study. Unpublished Bayer AG report No. 943045, dated 20 October 1997, from Novartis Crop Protection AG, Stein, Switzerland.

Klaassen, C.D. ed. (1996) *Casarett and Doull's toxicology: the science of poisons*. Fifth Ed., McGraw-Hill, pp. 716.

Losco, P. & Harleman, H. (1992) Normal development, growth, and aging of the lymph node. In: Mohr, U., Dungworth, D.L. & Capen, C.C., *Pathobiology of the aging rat*, Vol. I, Washington DC: ILSI Press, pp. 49–73.

Magnusson, B. & Kligman, A.M. (1969) The identification of contact allergens by animal assay. The guinea pig maximisation test. *J. Invest. Dermatol.*, **52**, 268–276.

Marty, J.H. (1994) Skin sensitisation test in the guinea pig—maximisation test. Unpublished Bayer AG report No. 943047, dated 28 September 1994, from Ciba-Geigy Ltd, Stein, Switzerland.

Marty, J.H. (1995) Acute dermal toxicity in the rat. Unpublished Bayer AG report No. 943161, dated 21 February 1995, from Ciba-Geigy Ltd, Stein, Switzerland.

Muller, T. (1996) Absorption, distribution, and excretion of [glyoxyl-phenyl-U-^{14}C] and [trifluormethyl-phenyl-U-^{14}C]trifloxystrobin in the rat. Unpublished Bayer AG report No. 13/96, dated 29 August 1996, from Ciba-Geigy Ltd, Basel, Switzerland.

Oishi, Y., Yoshizawa, K., Suzuki, J., Makino, N., Hase, K., Yamauchi, K. & Tsubura, A. (1995) Spontaneously occurring mammary adenocarcinoma in a 10-week old female rat. *Toxicol. Pathol.*, **23**, 696–700.

Palmer, A.K. (1978) Developmental abnormalities: rabbits. In: *Pathology of laboratory animals*, Vol. II, Chapter 20, New York: Springer Verlag, p. 1855.

Persohn, E. (1995a) Assessment of replicative DNA synthesis in the course of a 3 month oral toxicity study in rats. Bayer AG unpublished report No. CB 94/61, dated 25 September 1995, from Ciba-Geigy Ltd, Basel, Switzerland.

Persohn, E. (1995b) Assessment of replicative DNA synthesis in the course of a 3 month range finding toxicity study in mice. Unpublished Bayer AG report No. CB 94/60, dated 25 September 1995, from Ciba-Geigy Ltd, Basel, Switzerland.

Rumbeli, R. (1997a) The metabolism of [trifluormethyl-phenyl-U-^{14}C] trifloxystrobin after multiple oral administration to lactating goats. Unpublished Bayer AG report No. 09/97, dated 27 August 1997, from Novartis Crop Protection AG, Basel, Switzerland.

Rumbeli, R. (1997b) The metabolism of [glyoxyl-phenyl-U-^{14}C] trifloxystrobin after multiple oral administration to lactating goats. Unpublished Bayer AG report No. 14/97, dated 9 January 1997, from Novartis Crop Protection AG, Basel, Switzerland.

Stampf, P. (1998) Absorption, distribution, and excretion of [trifluormethyl-phenyl-U-^{14}C] and [glyoxyl-phenyl-U-^{14}C] trifloxystrobin in the rat (extension). Unpublished Bayer AG report No. 20/97, dated 8 January 1998, from Novartis Crop Protection AG, Basel, Switzerland.

Thanei, P. (1997) The metabolism of [glyoxyl-phenyl-U-^{14}C] and [trifluormethyl-phenyl-U-^{14}C] trifloxystrobin formulated as A-9604 A in the rat. Unpublished Bayer AG report No. 12/97, dated 14 November 1997, from Novartis Crop Protection AG, Basel, Switzerland.

Van de Sandt, J. (1997) In vitro percutaneous penetration of [glyoxyl-phenyl-U-^{14}C] trifloxystrobin formulated as A-9604 A through rat and human epidermis. Unpublished Bayer AG report No. 470956, dated 9 December 1997, from Novartis Crop Protection AG, Basel, Switzerland.

Winkler, G. (1996) Acute oral toxicity in the mouse (limit test). Unpublished Bayer AG report No. 963002, dated 18 March 1996, from Ciba-Geigy Ltd, Stein, Switzerland.

Winkler, G. (1997) Acute oral toxicity in the rat (limit test). Unpublished Bayer AG report No. 973006, dated 22 May 1997, from Ciba-Geigy Ltd, Stein, Switzerland.

ANNEX 1

Reports and other documents resulting from previous Joint Meetings Of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and WHO Expert Groups on Pesticide Residues

1. Principles governing consumer safety in relation to pesticide residues. Report of a meeting of a WHO Expert Committee on Pesticide Residues held jointly with the FAO Panel of Experts on the Use of Pesticides in Agriculture. FAO Plant Production and Protection Division Report, No. PL/1961/11; WHO Technical Report Series, No. 240, 1962.
2. Evaluation of the toxicity of pesticide residues in food. Report of a Joint Meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues. FAO Meeting Report, No. PL/1963/13; WHO/Food Add./23, 1964.
3. Evaluation of the toxicity of pesticide residues in food. Report of the Second Joint Meeting of the FAO Committee on Pesticides in Agriculture and the WHO Expert Committee on Pesticide Residues. FAO Meeting Report, No. PL/1965/10; WHO/Food Add./26.65, 1965.
4. Evaluation of the toxicity of pesticide residues in food. FAO Meeting Report, No. PL/1965/10/1; WHO/Food Add./27.65, 1965.
5. Evaluation of the hazards to consumers resulting from the use of fumigants in the protection of food. FAO Meeting Report, No. PL/1965/10/2; WHO/Food Add./28.65, 1965.
6. Pesticide residues in food. Joint report of the FAO Working Party on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies, No. 73; WHO Technical Report Series, No. 370, 1967.
7. Evaluation of some pesticide residues in food. FAO/PL:CP/15; WHO/Food Add./67.32, 1967.
8. Pesticide residues. Report of the 1967 Joint Meeting of the FAO Working Party and the WHO Expert Committee. FAO Meeting Report, No. PL:1967/M/11; WHO Technical Report Series, No. 391, 1968.
9. 1967 Evaluations of some pesticide residues in food. FAO/PL:1967/M/11/1; WHO/Food Add./68.30, 1968.
10. Pesticide residues in food. Report of the 1968 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies, No. 78; WHO Technical Report Series, No. 417, 1968.
11. 1968 Evaluations of some pesticide residues in food. FAO/PL:1968/M/9/1; WHO/Food Add./69.35, 1969.
12. Pesticide residues in food. Report of the 1969 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Group on Pesticide Residues. FAO Agricultural Studies, No. 84; WHO Technical Report Series, No. 458, 1970.
13. 1969 Evaluations of some pesticide residues in food. FAO/PL:1969/M/17/1; WHO/Food Add./70.38, 1970.
14. Pesticide residues in food. Report of the 1970 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies, No. 87; WHO Technical Report Series, No. 4574, 1971.
15. 1970 Evaluations of some pesticide residues in food. AGP:1970/M/12/1; WHO/Food Add./71.42, 1971.
16. Pesticide residues in food. Report of the 1971 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies, No. 88; WHO Technical Report Series, No. 502, 1972.
17. 1971 Evaluations of some pesticide residues in food. AGP:1971/M/9/1; WHO Pesticide Residue Series, No. 1, 1972.
18. Pesticide residues in food. Report of the 1972 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies, No. 90; WHO Technical Report Series, No. 525, 1973.
19. 1972 Evaluations of some pesticide residues in food. AGP:1972/M/9/1; WHO Pesticide Residue Series, No. 2, 1973.
20. Pesticide residues in food. Report of the 1973 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies, No. 92; WHO Technical Report Series, No. 545, 1974.
21. 1973 Evaluations of some pesticide residues in food. FAO/AGP/1973/M/9/1; WHO Pesticide Residue Series, No. 3, 1974.
22. Pesticide residues in food. Report of the 1974 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Agricultural Studies, No. 97; WHO Technical Report Series, No. 574, 1975.
23. 1974 Evaluations of some pesticide residues in food. FAO/AGP/1974/M/11; WHO Pesticide Residue Series, No. 4, 1975.

24. Pesticide residues in food. Report of the 1975 Joint Meeting of the FAO Working Party of Experts on Pesticide Residues and the WHO Expert Committee on Pesticide Residues. FAO Plant Production and Protection Series, No. 1; WHO Technical Report Series, No. 592, 1976.
25. 1975 Evaluations of some pesticide residues in food. AGP:1975/M/13; WHO Pesticide Residue Series, No. 5, 1976.
26. Pesticide residues in food. Report of the 1976 Joint Meeting of the FAO Panel of Experts on Pesticide Residues and the Environment and the WHO Expert Group on Pesticide Residues. FAO Food and Nutrition Series, No. 9; FAO Plant Production and Protection Series, No. 8; WHO Technical Report Series, No. 612, 1977.
27. 1976 Evaluations of some pesticide residues in food. AGP:1976/M/14, 1977.
28. Pesticide residues in food—1977. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues and Environment and the WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 10 Rev, 1978.
29. Pesticide residues in food: 1977 evaluations. FAO Plant Production and Protection Paper 10 Suppl., 1978.
30. Pesticide residues in food—1978. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues and Environment and the WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 15, 1979.
31. Pesticide residues in food: 1978 evaluations. FAO Plant Production and Protection Paper 15 Suppl., 1979.
32. Pesticide residues in food—1979. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 20, 1980.
33. Pesticide residues in food: 1979 evaluations. FAO Plant Production and Protection Paper 20 Suppl., 1980.
34. Pesticide residues in food—1980. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 26, 1981.
35. Pesticide residues in food: 1980 evaluations. FAO Plant Production and Protection Paper 26 Suppl., 1981.
36. Pesticide residues in food—1981. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 37, 1982.
37. Pesticide residues in food: 1981 evaluations. FAO Plant Production and Protection Paper 42, 1982.
38. Pesticide residues in food—1982. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 46, 1982.
39. Pesticide residues in food: 1982 evaluations. FAO Plant Production and Protection Paper 49, 1983.
40. Pesticide residues in food—1983. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 56, 1985.
41. Pesticide residues in food: 1983 evaluations. FAO Plant Production and Protection Paper 61, 1985.
42. Pesticide residues in food—1984. Report of the Joint Meeting on Pesticide Residues. FAO Plant Production and Protection Paper 62, 1985.
43. Pesticide residues in food—1984 evaluations. FAO Plant Production and Protection Paper 67, 1985.
44. Pesticide residues in food—1985. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 68, 1986.
45. Pesticide residues in food—1985 evaluations. Part I. Residues. FAO Plant Production and Protection Paper 72/1, 1986.
46. Pesticide residues in food—1985 evaluations. Part II. Toxicology. FAO Plant Production and Protection Paper 72/2, 1986.
47. Pesticide residues in food—1986. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 77, 1986.
48. Pesticide residues in food—1986 evaluations. Part I. Residues. FAO Plant Production and Protection Paper 78, 1986.
49. Pesticide residues in food—1986 evaluations. Part II. Toxicology. FAO Plant Production and Protection Paper 78/2, 1987.
50. Pesticide residues in food—1987. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 84, 1987.
51. Pesticide residues in food—1987 evaluations. Part I. Residues. FAO Plant Production and Protection Paper 86/1, 1988.
52. Pesticide residues in food—1987 evaluations. Part II. Toxicology. FAO Plant Production and Protection Paper 86/2, 1988.
53. Pesticide residues in food—1988. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 92, 1988.

54. Pesticide residues in food—1988 evaluations. Part I. Residues. FAO Plant Production and Protection Paper 93/1, 1988.
55. Pesticide residues in food—1988 evaluations. Part II. Toxicology. FAO Plant Production and Protection Paper 93/2, 1989.
56. Pesticide residues in food—1989. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 99, 1989.
57. Pesticide residues in food—1989 evaluations. Part I. Residues. FAO Plant Production and Protection Paper 100, 1990.
58. Pesticide residues in food—1989 evaluations. Part II. Toxicology. FAO Plant Production and Protection Paper 100/2, 1990.
59. Pesticide residues in food—1990. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 102, Rome, 1990.
60. Pesticide residues in food—1990 evaluations. Part I. Residues. FAO Plant Production and Protection Paper 103/1, Rome, 1990.
61. Pesticide residues in food—1990 evaluations. Part II. Toxicology. World Health Organization, WHO/PCS/91.47, Geneva, 1991.
62. Pesticide residues in food—1991. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 111, Rome, 1991.
63. Pesticide residues in food—1991 evaluations. Part I. Residues. FAO Plant Production and Protection Paper 113/1, Rome, 1991.
64. Pesticide residues in food—1991 evaluations. Part II. Toxicology. World Health Organization, WHO/PCS/92.52, Geneva, 1992.
65. Pesticide residues in food—1992. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 116, Rome, 1993.
66. Pesticide residues in food—1992 evaluations. Part I. Residues. FAO Plant Production and Protection Paper 118, Rome, 1993.
67. Pesticide residues in food—1992 evaluations. Part II. Toxicology. World Health Organization, WHO/PCS/93.34, Geneva, 1993.
68. Pesticide residues in food—1993. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 122, Rome, 1994.
69. Pesticide residues in food—1993 evaluations. Part I. Residues. FAO Plant Production and Protection Paper 124, Rome, 1994.
70. Pesticide residues in food—1993 evaluations. Part II. Toxicology. World Health Organization, WHO/PCS/94.4, Geneva, 1994.
71. Pesticide residues in food—1994. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and a WHO Expert Group on Pesticide Residues. FAO Plant Production and Protection Paper 127, Rome, 1995.
72. Pesticide residues in food—1994 evaluations. Part I. Residues. FAO Plant Production and Protection Paper 131/1 and 131/2 (2 volumes), Rome, 1995.
73. Pesticide residues in food—1994 evaluations. Part II. Toxicology. World Health Organization, WHO/PCS/95.2, Geneva, 1995.
74. Pesticide residues in food—1995. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the Core Assessment Group. FAO Plant Production and Protection Paper 133, Rome, 1996.
75. Pesticide residues in food—1995 evaluations. Part I. Residues. FAO Plant Production and Protection Paper 137, 1996.
76. Pesticide residues in food—1995 evaluations. Part II. Toxicological and Environmental. World Health Organization, WHO/PCS/96.48, Geneva, 1996.
77. Pesticide residues in food—1996. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. FAO Plant Production and Protection Paper, 140, 1997.
78. Pesticide residues in food—1996 evaluations. Part I. Residues. FAO Plant Production and Protection Paper, 142, 1997.
79. Pesticide residues in food—1996 evaluations. Part II. Toxicological. World Health Organization, WHO/PCS/97.1, Geneva, 1997.
80. Pesticide residues in food—1997. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. FAO Plant Production and Protection Paper, 145, 1998.

81. Pesticide residues in food—1997 evaluations. Part I. Residues. FAO Plant Production and Protection Paper, 146, 1998.
82. Pesticide residues in food—1997 evaluations. Part II. Toxicological and Environmental. World Health Organization, WHO/PCS/98.6, Geneva, 1998.
83. Pesticide residues in food—1998. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. FAO Plant Production and Protection Paper, 148, 1999.
84. Pesticide residues in food—1998 evaluations. Part I. Residues. FAO Plant Production and Protection Paper, 152/1 and 152/2 (two volumes).
85. Pesticide residues in food—1998 evaluations. Part II. Toxicological and Environmental. World Health Organization, WHO/PCS/99.18, Geneva, 1999.
86. Pesticide residues in food—1999. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. FAO Plant Production and Protection Paper, 153, 1999.
87. Pesticide residues in food—1999 evaluations. Part I. Residues. FAO Plant Production and Protection Paper, 157, 2000.
88. Pesticide residues in food—1999 evaluations. Part II. Toxicological. World Health Organization, WHO/PCS/00.4, Geneva, 2000.
89. Pesticide residues in food—2000. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. FAO Plant Production and Protection Paper, 163, 2001.
90. Pesticide residues in food—2000 evaluations. Part I. Residues. FAO Plant Production and Protection Paper, 165, 2001.
91. Pesticide residues in food—2000 evaluations. Part II. Toxicological. World Health Organization, WHO/PCS/01.3, 2001.
92. Pesticide residues in food—2001. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. FAO Plant Production and Protection Paper, 167, 2001.
93. Pesticide residues in food—2001 evaluations. Part I. Residues. FAO Plant Production and Protection Paper, 171, 2002.
94. Pesticide residues in food—2001 evaluations. Part II. Toxicological. World Health Organization, WHO/PCS/02.1, 2002.
95. Pesticide residues in food—2002. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. FAO Plant Production and Protection Paper, 172, 2002.
96. Pesticide residues in food—2002 evaluations. Part I. Residues. FAO Plant Production and Protection Paper 175/1 and 175/2 (two volumes).
97. Pesticide residues in food—2002 evaluations. Part II. Toxicological. World Health Organization, WHO/PCS/03.1, 2003.
98. Pesticide residues in food—2003. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. FAO Plant Production and Protection Paper, 176, 2004.
99. Pesticide residues in food—2003 evaluations. Part I. Residues. FAO Plant Production and Protection Paper, 177, 2004.
100. Pesticide residues in food—2003 evaluations. Part II. Toxicological. World Health Organization, WHO/PCS/04.1, 2004.
101. Pesticide residues in food—2004. Report of the Joint Meeting of the FAO Panel of Experts on Pesticide Residues in Food and the Environment and the WHO Core Assessment Group. FAO Plant Production and Protection Paper, 178, 2004.

This volume contains toxicological monographs that were prepared by the 2004 Joint FAO/WHO Meeting on Pesticide Residues (JMPR), which met in Rome from 20–29 September, 2004.

The monographs in this volume summarize the safety data on 14 pesticides that could leave residues in food commodities. These pesticides are bentazone, captan, dimethipin, fenpropimorph, fenpyroximate, fludioxinil, folpet, glyphosate, phorate, pirimicarb, propiconazole, triademefon/triademenol and trifloxystrobin. The data summarized in the toxicological monographs served as the basis for the acceptable daily intakes and acute reference doses that were established by the Meeting.

This volume and previous volumes of JMPR toxicological evaluations, many of which were published in the FAO Plant Production and Protection Paper series, contain information that is useful to companies that produce pesticides, government regulatory officers, industrial testing laboratories, toxicological laboratories and universities.

ISBN 92 4 166520 3