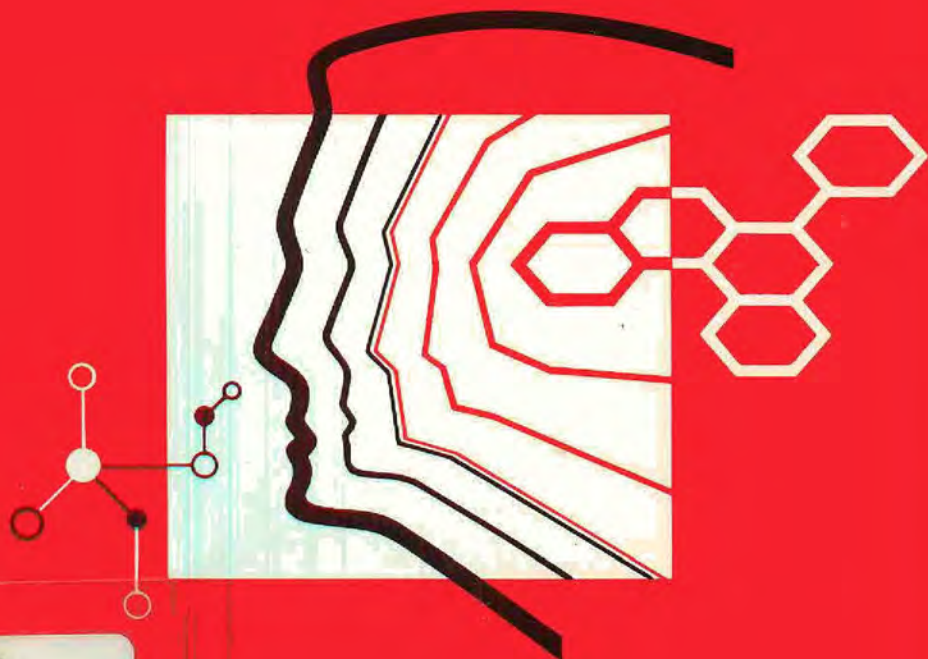


IPCS

INTERNATIONAL PROGRAMME ON CHEMICAL SAFETY

Environmental Health Criteria 183

Chlorothalonil



Under the joint sponsorship of the United Nations Environment Programme, the International Labour Organization and the World Health Organization, and produced within the framework of the International Organization Programme for the Sound Management of Chemicals

THE ENVIRONMENTAL HEALTH CRITERIA SERIES

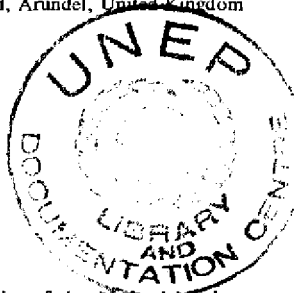
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This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the United Nations Environment Programme, the International Labour Organisation, or the World Health Organization.

Environmental Health Criteria 183

CHLOROTHALONIL

First draft prepared by Dr M.H. Litchfield, Arundel, United Kingdom



Published under the joint sponsorship of the United Nations Environment Programme, the International Labour Organisation, and the World Health Organization, and produced within the framework of the Inter-Organization Programme for the Sound Management of Chemicals.



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The **International Programme on Chemical Safety (IPCS)**, established in 1980, is a joint venture of the United Nations Environment Programme (UNEP), the International Labour Organisation (ILO), and the World Health Organization (WHO). The overall objectives of the IPCS are to establish the scientific basis for assessment of the risk to human health and the environment from exposure to 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, ILO, 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 UN Conference on 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.

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NOTE TO READERS OF THE CRITERIA MONOGRAPHS

Every effort has been made to present information in the criteria monographs as accurately as possible without unduly delaying their publication. In the interest of all users of the Environmental Health Criteria monographs, readers are requested to communicate any errors that may have occurred to the Director of the International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland, in order that they may be included in corrigenda.

* * *

A detailed data profile and a legal file can be obtained from the International Register of Potentially Toxic Chemicals, Case postale 356, 1219 Châtelaine, Geneva, Switzerland (Telephone No. 9799111).

* * *

This publication was made possible by grant number 5 U01 ES02617-15 from the National Institute of Environmental Health Sciences, National Institutes of Health, USA, and by financial support from the European Commission.

Environmental Health Criteria

P R E A M B L E

Objectives

In 1973 the WHO Environmental Health Criteria Programme was initiated with the following objectives:

- (i) to assess information on the relationship between exposure to environmental pollutants and human health, and to provide guidelines for setting exposure limits;
- (ii) to identify new or potential pollutants;
- (iii) to identify gaps in knowledge concerning the health effects of pollutants;
- (iv) to promote the harmonization of toxicological and epidemiological methods in order to have internationally comparable results.

The first Environmental Health Criteria (EHC) monograph, on mercury, was published in 1976 and since that time an ever-increasing number of assessments of chemicals and of physical effects have been produced. In addition, many EHC monographs have been devoted to evaluating toxicological methodology, e.g., for genetic, neurotoxic, teratogenic and nephrotoxic effects. Other publications have been concerned with epidemiological guidelines, evaluation of short-term tests for carcinogens, biomarkers, effects on the elderly and so forth.

Since its inauguration the EHC Programme has widened its scope, and the importance of environmental effects, in addition to health effects, has been increasingly emphasized in the total evaluation of chemicals.

The original impetus for the Programme came from World Health Assembly resolutions and the recommendations of the 1972 UN Conference on the Human Environment. Subsequently the work became an integral part of the International Programme on Chemical Safety (IPCS), a cooperative programme of UNEP, ILO and WHO. In this manner, with the strong support of the new partners, the importance of occupational health and environmental

effects was fully recognized. The EHC monographs have become widely established, used and recognized throughout the world.

The recommendations of the 1992 UN Conference on Environment and Development and the subsequent establishment of the Intergovernmental Forum on Chemical Safety with the priorities for action in the six programme areas of Chapter 19, Agenda 21, all lend further weight to the need for EHC assessments of the risks of chemicals.

Scope

The criteria monographs are intended to provide critical reviews on the effect on human health and the environment of chemicals and of combinations of chemicals and physical and biological agents. As such, they include and review studies that are of direct relevance for the evaluation. However, they do not describe *every* study carried out. Worldwide data are used and are quoted from original studies, not from abstracts or reviews. Both published and unpublished reports are considered and it is incumbent on the authors to assess all the articles cited in the references. Preference is always given to published data. Unpublished data are only used when relevant published data are absent or when they are pivotal to the risk assessment. A detailed policy statement is available that describes the procedures used for unpublished proprietary data so that this information can be used in the evaluation without compromising its confidential nature (WHO (1990) Revised Guidelines for the Preparation of Environmental Health Criteria Monographs. PCS/90.69, Geneva, World Health Organization).

In the evaluation of human health risks, sound human data, whenever available, are preferred to animal data. Animal and *in vitro* studies provide support and are used mainly to supply evidence missing from human studies. It is mandatory that research on human subjects is conducted in full accord with ethical principles, including the provisions of the Helsinki Declaration.

The EHC monographs are intended to assist national and international authorities in making risk assessments and subsequent risk management decisions. They represent a thorough evaluation of risks and are not, in any sense, recommendations for regulation or standard setting. These latter are the exclusive purview of national and regional governments.

Content

The layout of EHC monographs for chemicals is outlined below.

- Summary - a review of the salient facts and the risk evaluation of the chemical
- Identity - physical and chemical properties, analytical methods
- Sources of exposure
- Environmental transport, distribution and transformation
- Environmental levels and human exposure
- Kinetics and metabolism in laboratory animals and humans
- Effects on laboratory mammals and *in vitro* test systems
- Effects on humans
- Effects on other organisms in the laboratory and field
- Evaluation of human health risks and effects on the environment
- Conclusions and recommendations for protection of human health and the environment
- Further research
- Previous evaluations by international bodies, e.g., IARC, JECFA, JMPR

Selection of chemicals

Since the inception of the EHC Programme, the IPCS has organized meetings of scientists to establish lists of priority chemicals for subsequent evaluation. Such meetings have been held in: Ispra, Italy, 1980; Oxford, United Kingdom, 1984; Berlin, Germany, 1987; and North Carolina, USA, 1995. The selection of chemicals has been based on the following criteria: the existence of scientific evidence that the substance presents a hazard to human health and/or the environment; the possible use, persistence, accumulation or degradation of the substance shows that there may be significant human or environmental exposure; the size and nature of populations at risk (both human and other species) and risks for environment; international concern, i.e. the substance is of major interest to several countries; adequate data on the hazards are available.

If an EHC monograph is proposed for a chemical not on the priority list, the IPCS Secretariat consults with the Cooperating Organizations and all the Participating Institutions before embarking on the preparation of the monograph.

Procedures

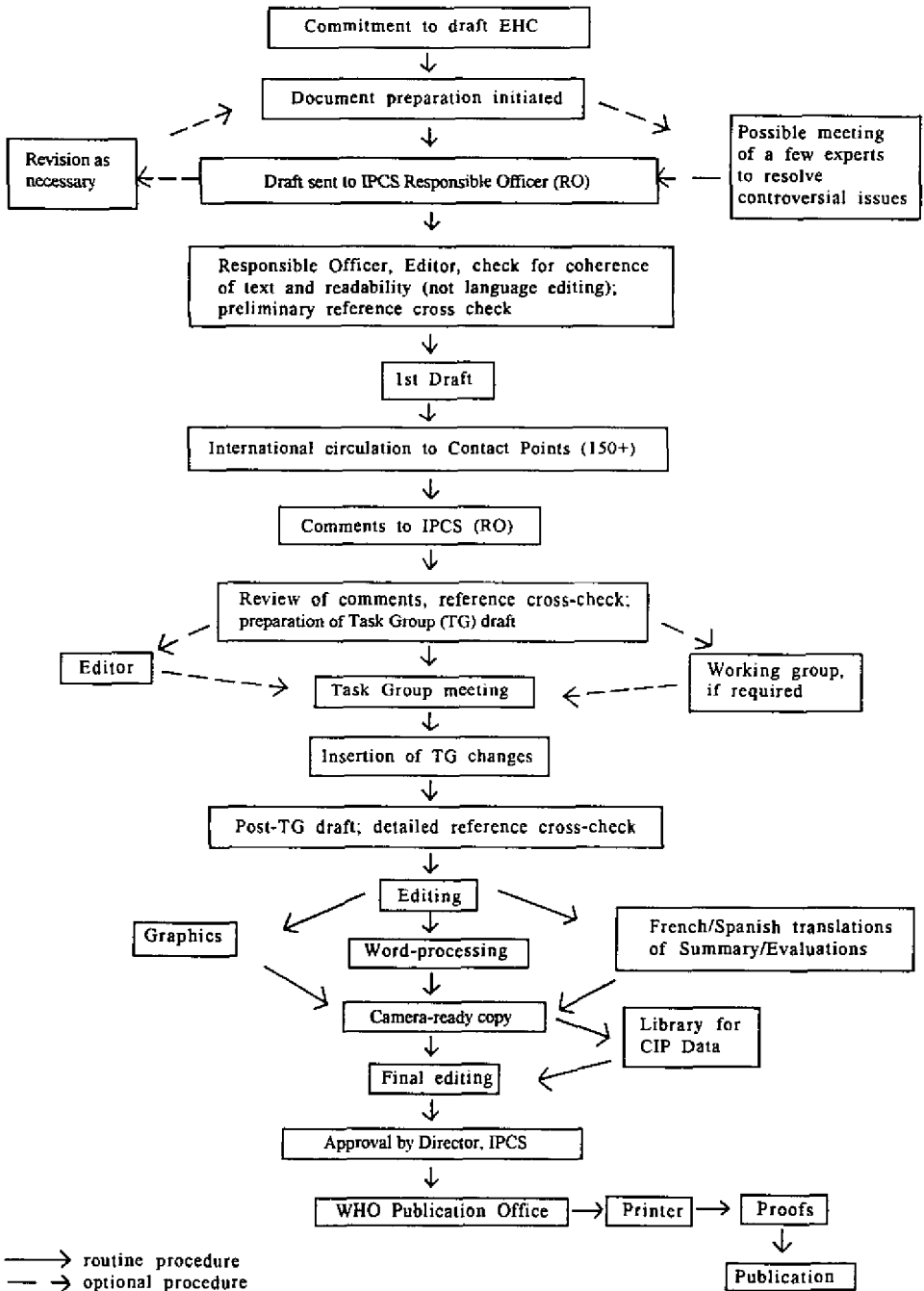
The order of procedures that result in the publication of an EHC monograph is shown in the flow chart. A designated staff member of IPCS, responsible for the scientific quality of the document, serves as Responsible Officer (RO). The IPCS Editor is responsible for layout and language. The first draft, prepared by consultants or, more usually, staff from an IPCS Participating Institution, is based initially on data provided from the International Register of Potentially Toxic Chemicals, and reference data bases such as Medline and Toxline.

The draft document, when received by the RO, may require an initial review by a small panel of experts to determine its scientific quality and objectivity. Once the RO finds the document acceptable as a first draft, it is distributed, in its unedited form, to well over 150 EHC contact points throughout the world who are asked to comment on its completeness and accuracy and, where necessary, provide additional material. The contact points, usually designated by governments, may be Participating Institutions, IPCS Focal Points, or individual scientists known for their particular expertise. Generally some four months are allowed before the comments are considered by the RO and author(s). A second draft incorporating comments received and approved by the Director, IPCS, is then distributed to Task Group members, who carry out the peer review, at least six weeks before their meeting.

The Task Group members serve as individual scientists, not as representatives of any organization, government or industry. Their function is to evaluate the accuracy, significance and relevance of the information in the document and to assess the health and environmental risks from exposure to the chemical. A summary and recommendations for further research and improved safety aspects are also required. The composition of the Task Group is dictated by the range of expertise required for the subject of the meeting and by the need for a balanced geographical distribution.

The three cooperating organizations of the IPCS recognize the important role played by nongovernmental organizations. Representatives from relevant national and international associations may be invited to join the Task Group as observers. While observers may provide a valuable contribution to the process, they can only speak at the invitation of the Chairperson.

EHC PREPARATION FLOW CHART



Observers do not participate in the final evaluation of the chemical; this is the sole responsibility of the Task Group members. When the Task Group considers it to be appropriate, it may meet *in camera*.

All individuals who as authors, consultants or advisers participate in the preparation of the EHC monograph must, in addition to serving in their personal capacity as scientists, inform the RO if at any time a conflict of interest, whether actual or potential, could be perceived in their work. They are required to sign a conflict of interest statement. Such a procedure ensures the transparency and probity of the process.

When the Task Group has completed its review and the RO is satisfied as to the scientific correctness and completeness of the document, it then goes for language editing, reference checking, and preparation of camera-ready copy. After approval by the Director, IPCS, the monograph is submitted to the WHO Office of Publications for printing. At this time a copy of the final draft is sent to the Chairperson and Rapporteur of the Task Group to check for any errors.

It is accepted that the following criteria should initiate the updating of an EHC monograph: new data are available that would substantially change the evaluation; there is public concern for health or environmental effects of the agent because of greater exposure; an appreciable time period has elapsed since the last evaluation.

All Participating Institutions are informed, through the EHC progress report, of the authors and institutions proposed for the drafting of the documents. A comprehensive file of all comments received on drafts of each EHC monograph is maintained and is available on request. The Chairpersons of Task Groups are briefed before each meeting on their role and responsibility in ensuring that these rules are followed.

**WHO TASK GROUP ON ENVIRONMENTAL HEALTH
CRITERIA FOR CHLOROTHALONIL**

Members

- Dr T. Bailey, US Environmental Protection Agency, Washington DC, USA
- Dr A.L. Black, Department of Human Services and Health, Canberra, Australia
- Mr D.J. Clegg, Carp, Ontario, Canada
- Dr S. Dobson, Institute of Terrestrial Ecology, Monks Wood, Abbots Ripton, Huntingdon, Cambridgeshire, United Kingdom (*Vice-Chairman*)
- Dr P.E.T. Douben, Her Majesty's Inspectorate of Pollution, London, United Kingdom (*EHC Joint Rapporteur*)
- Dr P. Fenner-Crisp, US Environmental Protection Agency, Washington DC, USA
- Dr R. Hailey, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, USA
- Ms K. Hughes, Environmental Health Directorate, Health Canada, Ottawa, Ontario, Canada (*EHC Joint Rapporteur*)
- Dr D. Kanungo, Central Insecticides Laboratory, Government of India, Ministry of Agriculture & Cooperation, Directorate of Plant Protection, Quarantine & Storage, Faridabad, Haryana, India
- Dr L. Landner, MFG, European Environmental Research Group Ltd, Stockholm, Sweden
- Dr M.H. Litchfield, Melrose Consultancy, Denmans Lane, Fontwell, Arundel, West Sussex, United Kingdom (*CAG Joint Rapporteur*)
- Professor M. Lotti, Institute of Occupational Medicine, University of Padua, Padua, Italy (*Chairman*)

-
- Professor D.R. Mattison, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, Pennsylvania, USA
- Dr J. Sekizawa, National Institute of Health Sciences, Tokyo, Japan
- Dr P. Sinhaseni, Chulalongkorn University, Bangkok, Thailand
- Dr S.A. Soliman, King Saud University, Bureidah, Saudi Arabia
- Dr M. Tasheva, National Centre of Hygiene, Medical Ecology and Nutrition, Sofia, Bulgaria (*CAG Joint Rapporteur*)
- Mr J.R. Taylor, Pesticides Safety Directorate, Ministry of Agriculture, Fisheries and Food, York, United Kingdom
- Dr H.M. Temmink, Wageningen Agricultural University, Wageningen, The Netherlands
- Dr M.I. Willems, TNO Nutrition and Food Research Institute, Zeist, The Netherlands

Representatives of GIFAP (Groupement International des Associations Nationales de Fabricants de Produits Agrochimiques)*

- Dr M. Bliss, Jr., ISK Biosciences Corporation, Mentor, Ohio, USA
- Dr A.C. Dykstra, Registration Department BPID, Solvay-Duphar BV, CP Weesp, The Netherlands
- Dr H. Frazier, ISK Biosciences Corporation, Mentor, Ohio, USA
- Dr R. Gardiner, GIFAP, Brussels, Belgium
- Dr B. Julin, Regulatory Affairs, Du Pont de Nemours (Belgium), Agricultural Products Department, Mercure Centre, Brussels, Belgium

* Participated as required for exchange of information.

- Dr S.M. Kennedy (Environmental Science), Du Pont de Nemours (Belgium), Agricultural Products Department, Mercure Centre, Brussels, Belgium
- Dr J. Killeen, ISK Biosciences Corporation, Mentor, Ohio, USA
- Dr Th. S.M. Koopman, Toxicology Department, Solvay-Duphar BV, CP Weesp, The Netherlands
- Dr R.L. Mull, Du Pont Agricultural Products, Wilmington, Delaware, USA
- Dr J.L.G. Thus, Environmental Research Department, Solvay-Duphar BV, CP Weesp, The Netherlands

Secretariat

- Ms A. Sundén Byléhn, International Register of Potentially Toxic Chemicals, United Nations Environment Programme, Châtelaine, Switzerland
- Dr P. Chamberlain, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland
- Dr J. Herrman, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland
- Dr K. Jager, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland
- Dr P. Jenkins, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland
- Dr W. Kreisel, World Health Organization, Geneva, Switzerland
- Dr M. Mercier, International Programme on Chemical Safety, World Health Organization, Geneva, Switzerland
- Dr M.I. Mikheev, Occupational Health, World Health Organization, Geneva, Switzerland
- Dr G. Moy, Food Safety, World Health Organization, Geneva, Switzerland

**Mr I. Obadia, International Labour Organisation, Geneva,
Switzerland**

**Dr R. Pleština, International Programme on Chemical Safety,
World Health Organization, Geneva, Switzerland**

**Dr E. Smith, International Programme on Chemical Safety,
World Health Organization, Geneva, Switzerland
(*EHC Secretary*)**

**Mr J. Wilbourn, International Agency for Research on Cancer,
Lyon, France**

ENVIRONMENTAL HEALTH CRITERIA FOR CHLOROTHALONIL

The Core Assessment Group (CAG) of the Joint Meeting on Pesticides met in Geneva from 25 October to 3 November 1994. Dr W. Kreisel of the WHO welcomed the participants on behalf of WHO, and Dr M. Mercier, Director, IPCS, on behalf of the IPCS and its cooperating organizations (UNEP/ILO/WHO). The Group reviewed and revised the draft monograph and made an evaluation of the risks for human health and the environment from exposure to chlorothalonil.

The first draft of the monograph was prepared by Dr M.H. Litchfield, Arundel, United Kingdom. The second draft, incorporating comments received following circulation of the first draft to the IPCS contact points for Environmental Health Criteria monographs, was prepared by the IPCS Secretariat.

Dr K.W. Jager and Dr P.G. Jenkins, both members of the IPCS Central Unit, were responsible for the overall scientific content and technical editing, respectively.

The fact that ISK Biosciences Corporation made available to the IPCS its proprietary toxicological information on chlorothalonil is gratefully acknowledged. This allowed the CAG to make its evaluation on a more complete database.

The efforts of all who helped in the preparation and finalization of the monograph are gratefully acknowledged.

ABBREVIATIONS

BCF	bioconcentration factor
BUN	blood urea nitrogen
ECD	electron capture detector
EDB	1,2-dibromoethane (ethylene dibromide)
FID	flame ionization detector
GC	gas chromatography
GSH	glutathione
γ -GT	γ -glutamyltranspeptidase
HECD	Hall electron capture detector
LOEL	lowest-observed-effect level
MS	mass spectrometry
NADPH	reduced nicotinamide adenine dinucleotide phosphate
NOEL	no-observed-effect level
PIB	piperonyl butoxide
SGOT	serum glutamic-oxalic transaminase
SGPT	serum glutamic-pyruvic transaminase
TEAM	total exposure assessment methodology
TWA	time-weighted average
UDS	unscheduled DNA synthesis
VHH	volatile halogenated hydrocarbon
VOC	volatile organic carbon compound

1. SUMMARY AND EVALUATION; CONCLUSIONS AND RECOMMENDATIONS

1.1 Summary

1.1.1 *Identity, physical and chemical properties, and analytical methods*

Chlorothalonil is a colourless, odourless, crystalline solid with a melting point of 250 °C and a vapour pressure of 7.63×10^{-5} Pa (5.72×10^{-7} mmHg) at 25 °C. It has low water solubility (0.6-1.2 mg/litre at 25 °C) and an octanol/water partition coefficient ($\log K_{ow}$) of 2.882. It is hydrolysed in water slowly at pH 9 but is stable at pH 7 or below (at 25 °C).

The most prevalent analytical method, after sample extraction and clean-up, is gas-liquid chromatography using an electron-capture detector.

1.1.2 *Sources of human and environmental exposure*

Chlorothalonil has been produced commercially since 1969 by chlorination of isophthalonitrile or by treatment of tetrachloro-isophthaloyl amide with phosphorus oxychloride. It is a fungicide with a broad spectrum of activity used mainly in agriculture but also on turf, lawns and ornamental plants. Crops protected include pome and stone fruit, citrus, currants, berries, bananas, tomatoes, green vegetables, coffee, peanuts, potatoes, onions and cereals. In addition, it is used in wood preservatives and in paints.

The three main formulations are a suspension concentrate, a water dispersible granule and a wettable powder. They are readily diluted with water and applied by ground spray systems or by air. Typical active ingredient rates are 1.2-2.5 kg/ha for crops such as beans, celery and onions. The main sources of human exposure will be during preparation and application of the products and from ingestion of crop residues in foodstuff (see section 1.1.4).

1.1.3 *Environmental transport, distribution and transformation*

Chlorothalonil is removed from aqueous media by strong adsorption on suspended matter. Modelled data suggest little or no partition to bottom sediment. Biodegradation may occur in natural waters with enzyme processes being involved. Chlorothalonil is rapidly degraded in soil, and degradation may occur in water with

the production of the 4-hydroxy metabolite, 4-hydroxy-2,5,6-trichloroisophthalonitrile. Half-lives for dissipation of the 4-hydroxy metabolite in soils range between 6 and 43 days.

Chlorothalonil does not translocate from the site of application to other parts of a plant. It is metabolized only to a limited extent on plants and the 4-hydroxy metabolite is usually < 5% of the residue.

Chlorothalonil is metabolized in fish via glutathione conjugation to give more polar excretory products. The enzyme glutathione-S-transferase is involved with this conversion. High concentrations of radiolabel found in the gall bladder and bile, after exposure of rainbow trout to ¹⁴C-chlorothalonil, are consistent with the excretion of the compound as glutathione conjugates. The concentrations of radiolabel accumulating in the gall bladder and other organs fell rapidly when the fish were placed in clean water.

Chlorothalonil does not bioaccumulate in aquatic organisms.

1.1.4 Environmental levels and human exposure

In a potato crop study, a small stream was oversprayed with chlorothalonil. Subsequent sampling/analysis of down-stream water demonstrated rapid disappearance of chlorothalonil (i.e. 450 µg/litre at 30 min post-spraying to 2-6 µg/litre at 12 h post-spraying). The routine spraying of irrigated field crops such as potatoes and barley gave rise to low concentrations of chlorothalonil (0.04-3.6 µg/litre) in tile drain water on a small number of sampling occasions.

Crop residues are composed mainly of chlorothalonil itself. The residue levels are dependent upon the applied rate, time interval between the last application and harvest, and the type of crop. Residue levels at harvest can be derived from the numerous supervised trials that have taken place on many crops worldwide and reported to FAO/WHO. Residues of chlorothalonil in dairy products are expected to be undetectable or very low. Dairy cows given high concentrations (up to 250 mg/kg) of chlorothalonil in their feed for 30 days showed no detectable residue in milk and only very low levels in tissues.

Total diet and individual food analysis in several countries have shown undetectable or low concentrations of chlorothalonil in

sampling surveys. Residue levels on foodstuffs are further reduced by preparation processes such as washing, peeling and cooking.

1.1.5 Kinetics and metabolism in laboratory animals

About 30% of an oral dose of chlorothalonil is absorbed within 48 h in rats at doses up to 50 mg/kg body weight. At higher doses, absorption is lower, indicating a saturation process. When ¹⁴C-chlorothalonil is given orally the radioactivity is distributed into blood and tissues within 2 h. The greatest concentration is found in the kidney, followed by liver and blood. The kidneys contain 0.3% of a 5 mg/kg body weight dose after 24 h.

Most of an oral dose of chlorothalonil in rats is found in faeces (> 82% within 48-72 h, regardless of dose). Biliary excretion is rapid, peaking within 2 h after a 5 mg/kg body weight oral dose, and is saturated at 50 mg/kg body weight and above. Urinary excretion accounts for 5-10% of the dose in rats. Faecal excretion is the main route in dogs and monkeys but urinary excretion (< 4%) is less than in rats.

Metabolic studies in rats indicate that chlorothalonil is conjugated with glutathione in the liver as well as in the gastrointestinal tract. Some of the glutathione conjugates may be absorbed from the intestine and transported to the kidneys, where they are converted by cytosolic β -lyase to thiol analogues that are excreted in the urine. When germ-free rats are dosed with chlorothalonil, the thiol metabolites appear in urine in much smaller amounts than with normal rats, indicating the involvement of intestinal microflora in the metabolism of chlorothalonil. Dogs or monkeys dosed orally with chlorothalonil excrete little or no thiol derivatives in urine.

When ¹⁴C-chlorothalonil was applied to rat skin, approximately 28% of the dose was absorbed within 120 h. About 18% of the dose was found in faeces and 6% in urine within 120 h.

1.1.6 Effects on laboratory mammals and in vitro test systems

Chlorothalonil has low acute oral and dermal toxicity in rats and rabbits, respectively (acute oral and dermal LD₅₀ values are > 10 000 mg/kg body weight). Hammer-milled technical chlorothalonil (MMAD 5-8 μ m) exhibited high toxicity in rats in an inhalation study, with a 4-h LC₅₀ of 0.1 mg/litre.

Chlorothalonil is a skin and eye irritant in the rabbit. Skin sensitization studies in the guinea-pig were inconclusive.

The main effects of repeated oral dosing in rats are on the stomach and kidney. Groups of 25 rats of each sex per group were fed chlorothalonil at 0, 1.5, 3, 10 or 40 mg/kg body weight per day in the diet for 13 weeks, and this was followed by a 13-week recovery period. Increased incidences of hyperplasia and hyperkeratosis of the forestomach occurred at 10 and 40 mg/kg; these reversed when treatment ceased. At 40 mg/kg, there was an increased incidence of hyperplasia of kidney proximal tubular epithelium in males at 13 weeks and after the recovery period. The NOEL was 3 mg/kg body weight per day based upon lack of forestomach lesions. The onset of the forestomach and kidney changes was shown to be rapid, with the lesions developing within 4-7 days in male rats at a dietary level of 175 mg/kg body weight per day.

In a 13-week study on mice (0, 7.5, 15, 50, 275 or 750 mg/kg in the diet), increased incidences of hyperplasia and hyperkeratosis of the squamous epithelial cells of the forestomach occurred in males and females at 50 mg/kg diet and above. The NOEL, based upon these changes, was 15 mg/kg chlorothalonil in the diet, equivalent to 3 mg/kg body weight per day.

A 16-week study in dogs with dietary levels of 0, 250, 500 or 750 mg/kg showed no treatment-related changes.

The forestomach and kidney lesions were investigated further in 2-year studies on rats, mice and dogs. In a study on rats (0, 1.8, 3.8, 15 or 175 mg/kg body weight per day), the effects were characterized histologically as an increase in the incidence and severity of hyperplasia, hyperkeratosis, and ulcers and erosions of the squamous mucosa of the forestomach, and as epithelial hyperplasia of the kidney proximal convoluted tubules at 3.8 mg/kg and above. The NOEL for non-neoplastic effects was therefore 1.8 mg/kg. The incidence of renal tumours (adenomas and carcinomas) and forestomach tumours (papillomas and carcinomas) was markedly increased at 175 mg/kg. There was evidence for an increased incidence of kidney tumours in males at 15 mg/kg and of stomach tumours at 3.8 and 15 mg/kg in males and females. The NOEL for neoplastic effects was therefore 1.8 mg/kg body weight per day based upon changes in forestomach tumour incidence. Supporting evidence for the carcinogenic potential of chlorothalonil in the kidney and

forestomach of rats was provided by the results from other 2-year studies at higher dose levels.

In a study on mice (0, 15, 40, 175 or 750 mg/kg in the diet), an increased incidence of renal tubular hyperplasia occurred at 175 mg/kg and above and of hyperplasia and hyperkeratosis of the forestomach at 40 mg/kg and above. The incidence of squamous tumours of the forestomach was slightly increased at 750 mg/kg. The NOELs for neoplastic and non-neoplastic changes were therefore 175 and 15 mg/kg in the diet (equivalent to 17.5 and 1.6 mg/kg body weight per day, respectively). Supporting evidence for these effects in the mouse was provided in another study at higher dose levels, but a study in B6C3F₁ mice did not show any evidence for carcinogenic potential at high dose levels.

In a 2-year study on dogs (60 and 120 mg/kg in the diet), no effects attributable to chlorothalonil were found. The NOEL was therefore 120 mg/kg in the diet (equivalent to 3 mg/kg body weight per day).

Chlorothalonil was not mutagenic in several *in vitro* and *in vivo* tests, although it was positive in a small number of assays.

The monothio, dithio, trithio, dicysteine, tricysteine and monogluthathione derivatives of chlorothalonil, which are potential nephrotoxicants, were shown to be negative in the Ames assay.

Chlorothalonil was not teratogenic in rats or rabbits at doses up to 400 and 50 mg/kg body weight per day, respectively. Reproductive parameters such as mating, fertility and gestation length were not affected by chlorothalonil at levels up to 1500 mg/kg in the diet in a two-generation study in rats.

The acute oral toxicity of the 4-hydroxy metabolite is greater than that of chlorothalonil itself (acute oral LD₅₀ of 332 mg/kg body weight versus > 10 000 mg/kg body weight). Several studies have been undertaken to characterize the toxicological profile of this metabolite and to establish NOELs.

1.1.7 Effects on humans

Contact dermatitis has been reported for personnel working in chlorothalonil manufacturing and in farmers and horticultural workers. Workers in the manufacture of wood products have also developed contact dermatitis on the hands and face when wood preservatives containing chlorothalonil were used.

1.1.8 Effects on other organisms in the laboratory and field

Chlorothalonil is highly toxic to fish and aquatic invertebrates in laboratory studies, the LC_{50} values being below 0.5 mg/litre. The maximum acceptable toxicant concentration (MATC) in a two-generation reproduction study in *Daphnia magna* was 35 µg/litre.

With minor exceptions, chlorothalonil is not phytotoxic.

The LC_{50} of a suspension concentrate formulation (500 g chlorothalonil/litre) in artificial soil for earthworms was > 1000 mg/kg soil (14 days). Earwigs suffered increased mortality when in contact with chlorothalonil residues on peanut foliage or ingesting it as a food source in laboratory tests; there was no other indication of insecticidal action.

Chlorothalonil is of low toxicity to birds with a reported acute oral LD_{50} of 4640 mg/kg diet in the mallard duck. No significant reproductive effects were reported.

A field study of aquatic organisms exposed following chlorothalonil application suggests that the toxicity is less than that predicted from laboratory studies; this is again consistent with the physicochemical properties of the compound. Deaths were seen in some species exposed experimentally in the field. There have been no reported incidents of kills in the environment. However, despite the short residence time of chlorothalonil in environmental media, kills would be expected to occur. Linking kills to the compound would be difficult given that residues would not persist long enough for chlorothalonil to be identified.

1.2 Evaluation

1.2.1 Evaluation of human health risks

The review of the toxicological data for chlorothalonil revealed that the most important studies for human risk estimation were the long-term studies in rodents and dogs.

In the rodent studies, chlorothalonil caused lesions in the forestomach and kidney. The lesions in the forestomach were characterized as hyperplasia and hyperkeratosis of the squamous epithelial cells. These occurred soon after dosing and were shown to be reversible after dosing ceased. Long-term administration led

to the formation of tumours (papilloma and carcinoma). The renal lesions in rodents were of rapid onset and characterized as hyperplasia of the proximal tubular epithelium. On longer-term administration, renal tumours (adenoma and carcinoma) occurred in the rat and in one study on mice.

In order to interpret the significance of these findings, the results of the mutagenic studies were taken into account. Chlorothalonil gave negative results in *in vitro* and *in vivo* mutagenic assays in which a variety of end-points were studied. Thiol derivatives of chlorothalonil were negative in the Ames test, and ¹⁴C-chlorothalonil did not bind to rat kidney DNA *in vivo*. The compound does not appear to have genotoxic potential on this basis, indicating that it probably exerts its carcinogenic effect in rodents via a non-genotoxic mechanism. The initial forestomach lesions in rodents were attributed to the irritant action of chlorothalonil, and, where this does not occur, a NOEL can be attained. The irritant action on rodent forestomach in conjunction with the relatively long residence time of the compound in this organ were seen to be factors presenting the opportunity for the initiation of the lesions and leading to carcinogenic action on prolonged administration. It was concluded that, since humans do not possess a comparable organ, rodents are probably not representative of the action of this compound in man in this respect. This reasoning is also supported by the fact that another animal species, the dog, is not affected by the compound at similar or higher doses.

In the assessment of the relevance of the rodent renal lesions, the metabolic conversion of chlorothalonil to metabolites which act directly upon the kidney was seen to be a major factor. In the kidney glutathione conjugates are converted by β -lyase to chlorothalonil thiol derivatives. Chlorothalonil is thought to be conjugated with glutathione (GSH) mostly in the gastrointestinal tract prior to absorption, although there is evidence of glutathione conjugation at other sites. After absorption the conjugates pass to the kidney where they are converted to chlorothalonil thiol derivatives following the action of β -lyase. It has been shown *in vitro* that the di- and trithiol metabolites inhibit the function of renal cortical mitochondria. Therefore, a cycle of cell death and regenerative renal hyperplasia may be initiated.

In adducing the relevance of these findings for humans, the species differences in the metabolic pathway for chlorothalonil were taken into account. It was noted that the formation of the

thiol metabolites, as determined by urinary excretion, was considerably diminished when chlorothalonil was fed to germ-free rats. This indicates that the type and/or quantity of gut microflora has a determining role in the production of the thiol derivatives. Studies in dogs and monkeys showed that the excretion of the thiol derivatives was barely detectable after oral administration of chlorothalonil. This suggests that the rat is rather different from other species in this respect. Furthermore there is some evidence that β -lyase activity in the kidney varies among species, being an order of magnitude lower in humans than in rats.

For all the reasons stated above it was concluded that the rodent was not the most relevant species for evaluating the long-term effect of chlorothalonil in humans and that the dog was a more representative species for this purpose. The NOEL of 120 mg/kg in the diet in the 2-year study on dogs, equivalent to 3 mg/kg body weight per day, should therefore be used for the purpose of human risk estimation.

1.2.2 Evaluation of effects on the environment

Chlorothalonil is algicidal for a number of algal species. The fungicide does not inhibit bacterial growth except at very high concentrations in laboratory culture. Field and laboratory evidence shows no effects on nitrogen fixation or nitrification at recommended application rates and minimal effects at higher application rates in temperate soils. There was insufficient information to assess effects on the nitrogen cycle in tropical soils.

Laboratory acute toxicity tests show chlorothalonil to be very highly toxic to many aquatic animals including fish and *Daphnia*, although molluscs appear to be insensitive. The LC_{50} concentrations for a range of fish and invertebrates are similar and below 0.5 mg/litre.

A single study indicated reproductive effects in fish following continuous exposure for 35 days. Since the compound both adsorbs to suspended material and is degraded rapidly, the significance of this finding was considered to be questionable.

A field study of aquatic organisms exposed following chlorothalonil application suggests that the toxicity is less than that predicted from laboratory studies; this is again consistent with the physicochemical properties of the compound. Deaths were seen in

some species exposed experimentally in the field. There have been no reported incidents of kills in the environment. However, despite the short residence time of chlorothalonil in environmental media, kills would be expected to occur immediately after application. Linking kills to the compound would be difficult given that residues would not persist long enough for chlorothalonil to be identified.

With minor exceptions, chlorothalonil is not phytotoxic.

Several studies have shown no toxicity of chlorothalonil to earthworms at recommended application rates. At an exposure of five times the maximum recommended rate, the compound severely reduced worm reproduction.

Chlorothalonil is classified as "relatively non-toxic" to honeybees. Earwigs exposed to residues topically and via food showed some mortality (20-55%), but there is no other evidence of insecticidal action.

Chlorothalonil has low toxicity to birds in acute or dietary tests. The low acute toxicity of chlorothalonil to laboratory mammals tempered with its short persistence in the environment suggests minimal hazard to wild mammal species.

1.2.2.1 Transport, distribution and transformation

Chlorothalonil adsorbs strongly to organic matter in soil and suspended material in water. It is not, therefore, leached from soil to groundwater. It is removed rapidly from surface water to suspended material and to a lesser extent to bottom sediment. Chlorothalonil is not translocated in plants from the site of application.

Abiotic degradation of chlorothalonil in water through photolysis does not occur. Some hydrolysis does take place at higher pH.

Microbial degradation is the major cause of dissipation in soil and may take place to some extent in water; this involves several parallel processes, one of which leads to formation of the 4-hydroxy metabolite. Half-lives for dissipation of this metabolite from non-sterile soils range between 6 and 43 days. Biodegradation on plants is limited and the 4-hydroxy metabolite comprises less than 5% of the total residues.

During exposure, fish bioconcentrate chlorothalonil, but almost total degradation occurs within 2 weeks after termination of exposure. Chlorothalonil is metabolized in fish through glutathione conjugation and the conjugates are excreted through the bile.

1.2.2.2 Aquatic organisms

The results of a single field study measuring concentrations of chlorothalonil in water following overspray of the water were available; corresponding data on concentrations in suspended and bottom sediment were unreliable. Output from the EXAMS II fate model using the same application scenario produced estimated water concentrations which closely corresponded to the measured ones. Little or no chlorothalonil was predicted in bottom sediment.

Based on this combination of measured and modelled data, the ratio between a "toxic" concentration (the rainbow trout LC_{50}) and expected concentration is less than 1 for up to 5 h after overspray and increases rapidly thereafter. Similar results were obtained for daphnids. Therefore, despite its rapid removal from water and degradation, the high toxicity of chlorothalonil is expected to cause deaths of aquatic organisms in the period immediately after spraying. This is the worst case situation of direct water overspray.

There were no data to extend this quantitative evaluation to other field situations or climates.

1.2.2.3 Terrestrial organisms

A calculated maximum soil concentration, based on application of chlorothalonil at 2.5 kg a.i./ha and complete bioavailability, is 3 orders of magnitude higher than the lowest estimate of LC_{50} for earthworms.

For grazing birds (ducks and geese) total daily intake is at least a factor of 100 below the NOEL for oral toxicity. For rabbits, total daily intake is also at least 2 orders of magnitude lower than the reported NOEL. This is based on a maximum recommended application rate of 2.5 kg a.i./ha, an estimated worst case value for residues on grass, no degradation of the compound, consumption of the total daily intake at a single time and no choice but to eat contaminated food. Table 1 contains a summary of risk quotients for birds, fish and aquatic invertebrates.

Summary and Evaluation; Conclusions and Recommendations

Table 1. Toxicity/exposure ratios for birds, fish and aquatic invertebrates based on application rates of 2.5 kg a.i./ha of chlorothalonil to soybeans (worst case)

Risk category	LC ₅₀ (mg/litre or mg/kg diet)	Estimated exposure (mg/litre or mg/kg diet) ^{a,b}	Toxicity/exposure ratio (TER) ^c
Acute bird	4640	73.7-535.7	63.0-8.7
Acute fish (stream)	0.01	0.009-0.04	1.1-0.25
Acute fish (pond)	0.01	0.01	1.0
Acute aquatic invertebrate (stream)	0.07	0.009-0.04	7.8-1.8
Acute aquatic invertebrate (pond)	0.07	0.01	7.0

^a Estimated environmental concentration in the terrestrial environment (for bird exposure) is based on the stated application rate and the assumption of deposition on short grass using the US EPA nomogram.

^b Aquatic exposure concentrations were taken from the STREAM model based on a single application and estimated run-off into water; no direct overspray is included.

^c TER is the toxicity (as LC₅₀) divided by the exposure; values at or below 1.0 indicate likely exposure to toxic concentrations by organisms in the different risk categories.

1.2.3 Toxicological criteria for setting guidance values

The toxicological studies on chlorothalonil of relevance for setting guidance values are displayed in Table 2. The study results and their significance are described briefly and gaps in test requirements are indicated.

1.3 Conclusions and recommendations

Considering the toxicological characteristics of chlorothalonil, both qualitatively and quantitatively, it was concluded, on the basis of the NOEL of 3 mg/kg body weight per day derived in the 2-year study on dogs and applying a 100-fold uncertainty factor, that 0.03 mg/kg body weight per day will probably not cause adverse effects in humans by any route of exposure.

A study to assess the skin irritation potential is needed.

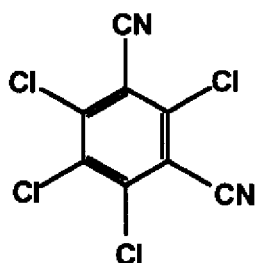
Table 2. Toxicological criteria for setting guidance values for chlorothalonil

Exposure scenario	Relevant route/effect/species	Result/remarks
Short-term (1-7 days)	skin, irritation, rabbit	irritant
	eye, irritation, rabbit	irritant
	skin, sensitization, guinea-pig	tests were inconclusive evidence in humans of contact dermatitis
	inhalation, lethality, rat	high toxicity in 4-h study with hammer-milled technical chlorothalonil (MMAD 5-8 μm); not relevant for most human exposure situations
Medium-term (1-26 weeks)	repeat dermal, rabbit	21-day study; irritant at 2.5 mg/kg body weight per day and above; no systemic effects at 50 mg/kg body weight per day
	repeat oral, mice and rats	13-22 week studies; NOEL = 3 mg/kg body weight per day in rats and mice
	maternal, oral, rabbit	teratology study; maternal toxicity NOEL = 10 mg/kg body weight per day by gavage; no fetotoxic or teratogenic effect
Long-term	repeat oral, dog	2-year study; NOEL = 3 mg/kg body weight per day

2. IDENTITY, PHYSICAL AND CHEMICAL PROPERTIES, ANALYTICAL METHODS

2.1 Identity

Chemical structure



Molecular formula	$C_8Cl_4N_2$
Relative molecular mass	265.9
CAS chemical name	2,4,5,6,-tetrachloro-1,3-benzenedi carbonitrile
CAS registry number	1897-45-6
RTECS registry number	NT2600000
Common name	chlorothalonil
IUPAC name	tetrachloroisophthalonitrile
Synonyms	m-TCPN; 2,4,5,6-tetrachloro-3-cyano benzonitrile
Trade names (manufacturers & suppliers)	Bravo (ISK Biotech) Daconil (ISK Biotech) Faber (Tripart Farm Chemicals) Repulse (ICI); Exotherm (Alto Elite) Nopocide (a preservative in paints and adhesives)
Technical product purity	> 97%

Technical product impurities (%) tetrachlorophthalonitrile (< 0.1), tetrachloroterephthalonitrile (0.1-1.6), pentachlorobenzonitrile (0.5-2.5), partially chlorinated dicyanobenzenes (0.2-1.0), unchlorinated dicyanobenzenes (0.1-1.6), HCB (0.03), insoluble in xylene (0.1-1.0)

2.2 Physical and chemical properties

The physical properties of chlorothalonil are listed in Table 3.

Table 3. Physical properties of chlorothalonil

Physical state	crystalline solid
Colour	colourless
Odour	odourless
Melting point (°C)	250-251
Boiling point (°C)	350 (760 mmHg)
Vapour pressure at 25 °C	5.72×10^{-7}
Relative density	1.8
Octanol-water partition coefficient (log K_{ow})	2.88-3.86
Solubility in water (mg/litre) at 25 °C	0.6-1.2
Solubility in organic solvents (g/litre)	acetone 20, dimethylformamide 30, dimethylsulfoxide 20, xylene 80, readily soluble in benzene

Chlorothalonil is non-flammable and non-explosive. It is thermally stable under normal storage conditions and to UV radiation, and it is chemically stable in neutral or acidic aqueous solutions. It breaks down at pH 9, the rate following first-order kinetics at 1.8% per day (at 25 °C) (Szalkowski & Stallard, 1977). It has been shown that chlorothalonil is unstable to light when dissolved in benzene and that 2,3,5-trichloro-4,6-dicyanobiphenyl

is a condensation product (Kawamura et al., 1978). Chlorothalonil is not corrosive.

2.3 Analytical methods

Analytical methods for determining chlorothalonil in formulations, fruit, vegetables, soil and water are summarized in Table 4. In general, the methods also detect the principal metabolite 4-hydroxy-2,5,6-trichloroisophthalonitrile.

2.3.1 Sample preparation

Samples are extracted initially with an organic solvent such as acetone. For samples where interference with the analytical method is expected, e.g., plant material, further partitioning with organic solvents is required, followed by clean-up on alumina or Florisil columns if necessary. The sample extracts are submitted for analytical determination.

2.3.2 Analytical determination

In most cases the cleaned-up sample extracts are analysed by gas-liquid chromatography using an electron capture detector. This provides sufficient sensitivity for the analysis of trace quantities of chlorothalonil residues at detection limits down to 0.01 mg/kg in many cases.

Where less sensitive determination is required, e.g., for formulation analysis, a flame ionization detector gives sufficient sensitivity. A method for formulation analysis using infrared spectroscopy after dichloromethane extraction has been reported (US EPA, 1976).

The Joint FAO/WHO Codex Alimentarius Commission has given recommendations for the methods of analysis to be used for the determination of chlorothalonil residues (FAO/WHO, 1989).

Table 4. Methods for the determination of chlorothalonil

Sample type	Sample preparation extraction/clean-up	Analytical method ^a	Limit of detection ($\mu\text{g}/\text{kg}$ or $\mu\text{g}/\text{litre}$)	Reference
Formulation	extract (1,4-dioxane or methyl ethyl ketone/ carbon disulfide/1,2-dimethoxyethane)	GC/TCD or GC/FID	-	Ballew et al. (1976)
Fruit & vegetable surfaces	Strip (dichloromethane) evaporate, dilute (benzene)	GC/ECD	10	Ballew et al. (1976)
Green leafy vegetables	extract (acidified acetone), evaporate, dissolve (aqueous NaHCO_3), adjust pH, extract (diisopropyl ether), evaporate, dilute (benzene), chromatograph (alumina)	GC/ECD	10	Ballew et al. (1976)
Fruit and vegetables	extract (acetone), evaporate, acidify and extract (ether), evaporate, chromatograph (Florisil), elute (acetone/dichloromethane)	GC/ECD	20	Burchfield & Stairs (1977)
Non-fatty products especially with S interference, onion, cabbage, celery	extract (toluene/isopropanol), aqueous separation, evaporate, chromatograph (alumina/ AgNO_3), elute (hexane)	GC/ECD	10-50	Holmes & Wood (1972)

Table 4 (contd).

Potatoes	extract (acidified acetone), chromatograph (Florisil) derivatize (diazomethane)	GC/ECD GC/MCD	10 20	Markus & Puma (1973)
Apples	rinse (acidified acetone), adjust pH, partition (hexane), extract tissue (acidified acetone), concentrate, partition (hexane), acidify aqueous fraction, partition (diisopropyl ether)	GC/ECD	50	Suzuki & Oda (1977)
Cranberries	extract (acetone), filler, (Celite 545), adsorb (Extrelut-20), elute (petroleum ether), evaporate, dissolve (benzene)	GC/ECD	not quoted	Carmoni et al. (1991)
Fresh fruit	extract (acetone), partition (petroleum ether and methylene chloride), concentrate	HPLC/UV (232 nm) and HPLC/photoconductivity detection (PC)	< 50	Gidvydis & Walters (1988)
Soil	extract (acidified acetone), extract (acetonitrile/hexane), partition (aqueous layer) extract (diisopropyl ether) concentrate, dilute (benzene) chromatograph (alumina)	GC/ECD	10	Ballew et al. (1976)
	extract (acetone: sulfuric acid), partition (petroleum ether), evaporate, redissolve in hexane/methylene chloride, elute, concentrate	GC/ECD	10	Kenyon & Wiedmann (1982b)

Table 4 (contd).

Sample type	Sample preparation extraction/clean-up	Analytical method ^a	Limit of detection (µg/kg or µg/litre)	Reference
Water	adjust pH to 4.5, extract (diisopropyl ether), concentrate, dilute (benzene)	GC/ECD	10	Ballec et al. (1976)
	adjust pH, extract (petroleum ether), add keeper, concentrate, redissolve (hexane/methylene chloride), elute (methylene chloride/hexane/acetone/nitrile)	GC/ECD	0.05	Kenyon & Wiedmann (1992a)
Air samples, dislodgeable residues	extraction (methional 2-propanol, <i>n</i> -hexane)	HPLC with UV detection at 254 or 325 nm	0.5	Jongen et al. (1991)

^a GC = gas chromatography; ECD = electron capture detector; FID = flame ionization detector; HPLC = high performance liquid chromatography; MCD = microcoulometric detection; TCD = thermal conductivity detection

3. SOURCES OF HUMAN AND ENVIRONMENTAL EXPOSURE

3.1 Natural occurrence

Chlorothalonil does not occur naturally in the environment.

3.2 Production levels and processes

Chlorothalonil is produced by the chlorination of isophthalonitrile or by treatment of tetrachloroisophthaloyl amide with phosphorus oxychloride. It has been produced commercially in the USA since 1969. No data on production are available but it has been estimated at 5000 tonnes annually (IARC, 1983). The annual production in Japan has been estimated to be 3000 tonnes (IARC, 1983).

Imports into the USA were 1650 tonnes in 1976 and 175 tonnes in 1980 (IARC, 1983).

No data are available on possible releases to the environment from production processes or transportation.

3.3 Uses

Chlorothalonil is a fungicide with a broad spectrum of activity used mainly in agriculture but also on turf, lawns and ornamental plants. It protects plants against a variety of fungal infections such as rusts, downy mildew, leaf spot, scabs, blossom blight and black pod. Crops protected include pome fruit, stone fruit, citrus, currants, cranberries, strawberries, bananas, vines, hops, tomatoes, green vegetables, tobacco, coffee, tea, soya bean, groundnuts, potatoes, onions, cereals and sugar beet. In addition, it is used in wood preservatives, fish net coatings and anti-fouling paints.

Global estimates of chlorothalonil use for these purposes are not available. The extent of use in various countries on an annual basis is shown in Table 5.

Chlorothalonil is used in agriculture in formulated products. The three main formulations are a suspension concentrate containing 500 g chlorothalonil/litre, a water dispersible granule and a wettable powder containing 75% chlorothalonil. The formulations mix readily with water and are diluted to give a spray

Table 5. Quantities of chlorothalonil used in various countries

Country	Year	Consumption (tonnes)	Usage	Reference
Canada (New Brunswick)	1982	5.1	potatoes	O'Neill (1991)
Colombia	1980	14.5	fruit, flowers, ornamentals	IRPTC (1989)
	1981	22.2	fruit, flowers, ornamentals	IRPTC (1989)
	1982	12.5	fruit, flowers, ornamentals	IRPTC (1989)
Mexico	1983	250	broccoli, potatoes, etc.	IRPTC (1989)
Sweden	1981	30	agricultural crops	IRPTC (1989)
		3	paint, wood	
Thailand	1976	6	agriculture	IRPTC (1989)
	1982	10.4		
United Republic of Tanzania	1981-2	640	coffee beans, tomatoes	IRPTC (1989)
USA	1976	2000	by farmers on major crops mildewicide in paint 53% peanuts, 31% vegetables, 12% turf, 5% potatoes	IARC (1983)
	1978	300		IARC (1983)
	1980	5000		IARC (1983)

mixture which can be applied by ground spray systems or by air, and as dilute or concentrated sprays.

The dose rates recommended for crop protection have been derived from efficacy studies conducted in a variety of climatic conditions in various parts of the world. The label recommendations are designed to give satisfactory fungal disease control and to keep residues within national and international limits. Typical active ingredient rates are 1.25-2.5 kg/ha for crops such as beans, celery and onions. Rates of use for a variety of purposes are shown in Table 6. Spray volumes usually range from about 200 to 400 litres/ha for dilute sprays and 45 to 95 litres/ha for concentrated sprays. Applications should commence when weather conditions favour disease, e.g., high humidity, and prior to initial infection. Repeat applications may be needed as directed on the label for the country concerned. Examples of crops, diseases controlled, agronomic importance, application rates, timing of treatment and pre-harvest intervals on a variety of crops in the Netherlands have been given by FAO (1982). A summary of approved uses for grapes, including formulation used, application rates, number of treatments and pre-harvest interval for a variety of countries, has been given by FAO/WHO (1986a).

Table 6. Ranges of application rates for chlorothalonil

	Application rate (kg active ingredient per ha)
Agronomic crops:	
Corn, lentils, peanuts, potatoes, soybeans, wheat, barley, rice	0.875-2.0
Tree fruit crops:	
Stone, citrus, nut, pome	1.25-3.5
Small fruit:	
Cranberry, blackberry, grape	1.25-5.85
Vegetable crops	0.875-2.5
Ornamentals	1.25-2.5
Turf	4.5-25.0

Chlorothalonil formulations are compatible in use with many other fungicides and insecticides and combined formulations are registered and available for use in many countries.

4. ENVIRONMENTAL TRANSPORT, DISTRIBUTION AND TRANSFORMATION

4.1 Transport and distribution between media

The sorptive characteristics of chlorothalonil have been investigated to estimate its potential for contamination of aquifers after application to a cranberry bog (Reduker et al., 1988). The soil studied was mainly sandy in character. The studies included a kinetic and an absorption equilibrium assessment, the soil being shaken with chlorothalonil in water for periods up to 48 h, and a soil column study with 2.8 mg chlorothalonil/100 ml at a flow rate of 642 ml/day for 64 days. A linear adsorption relationship was established with a partition coefficient for chlorothalonil of 74.4 ml/g for this soil. Very little (< 22%) of the adsorbed chemical was recovered. The soil column study produced a dispersion coefficient of 100 cm²/day. Only a small proportion (less than 2.8%) of chlorothalonil appeared in the effluent or was extracted from the soil, indicating either irreversible adsorption, degradation, or both.

The movement of chlorothalonil in a sandy soil was observed on a commercial farm with a high water table and a tile drain system in Manitoba, Canada. Chlorothalonil was routinely sprayed on irrigated crops such as potatoes and barley. In one season it was detected in the tile drain water on 4 out of 66 sampling days at concentrations of 0.04-3.66 µg/litre. In the same period chlorothalonil was also found in groundwater from a well on the site at levels of 10-272 µg/litre. There was some evidence of a small amount of carry-over into the following season (Krawchuk & Webster, 1987). They also reported serious background contamination problems due to the autosampler. When these problems were corrected (i.e., 1983), the residue levels in the well ranged from 0.9 to 8.6 µg/litre. In this report, the authors interpreted their data to demonstrate both leaching and potential carry-over. However, it should be noted that an initial tile water outflow sample, taken in 1981, showed no detectable chlorothalonil (i.e., < 0.02 µg/litre), although chlorothalonil was applied to the site that year.

Water/sediment measurements were made after aerial spraying of a potato crop in Canada (O'Neill, 1991). The area oversprayed included a small water course with a pond. The results showed a rapidly decreasing chlorothalonil content in the water phase after

overspraying, little or no compound being found in the sediment (63-91% sand). The author indicated that sediments with greater clay or silt content would play a greater role in chlorothalonil transport.

Analysis of stream water samples containing chlorothalonil showed significant binding to suspended material, with an average log partition coefficient ($\log P_{sm/w}$) of 5.695 and an average of 81% chlorothalonil being bound to the suspended matter. Algal growths on stream pebbles played a dominant role in chlorothalonil removal by absorption and biodegradation. It was also shown that *Galaxias auratus* enhanced chlorothalonil loss in fish tanks by a factor of 25 times (Davies, 1988).

Chlorothalonil does not translocate from the site of application to other parts of a plant. For example, ring-labelled ^{14}C -chlorothalonil does not translocate when applied topically to cucumber, bean or tomato leaves. It was not translocated into the aerial parts of corn or tomato plants when they were cultivated for 23 days in soil treated with ^{14}C -chlorothalonil. There was no movement or translocation of radioactivity within the root systems of sweet corn, cucumber or tomato grown in soil treated with ring-labelled chlorothalonil. This also indicated that the major 4-hydroxy metabolite in soil was not translocated (Kunkel, 1967a,b).

Chlorothalonil residues remaining on food crops at harvest may enter the human food chain. Residues in foodstuffs may be further reduced by processing and cooking (see sections 5.1 and 5.2).

4.2 Transformation

4.2.1 Biodegradation

Studies with river water from two sources in Tasmania showed that loss of chlorothalonil was slow in still water. Comparison of loss rates at 5 and 15 °C indicated involvement of enzymic processes. Uptake by algal growths also indicated biodegradation with the appearance of polar metabolites. However, biodegradation is unlikely to play a major role in the fate of chlorothalonil in moderate to fast flowing streams, where volatilization and adsorption are liable to be dominant factors (Davies, 1988).

Chlorothalonil is rapidly degraded in soil under both laboratory and field conditions. In laboratory experiments its half-life

ranged from 4 to 40 days in various types of soil. The rate increased with increasing organic matter content, moisture and temperature. It appeared that little was lost due to volatilization. On turf plots at three locations in the USA, the half-life of chlorothalonil ranged from 26 to 45 days after treatments (Stallard & Wolfe, 1967). The major soil degradation product is the 4-hydroxy metabolite, 4-hydroxy-2,5,6-trichloroisophthalonitrile. Laboratory studies in five soils showed half-lives for the 4-hydroxy metabolite of 36 days in a sandy loam and up to 220 days in clay type soil (Wolfe & Stallard, 1968). It has been shown that bacteria isolated from soil are capable of metabolizing chlorothalonil in culture media. It can be deduced that soil microorganisms play a role in the rapid degradation of chlorothalonil in soil (Duane, 1970).

Degradation of chlorothalonil in soil involves a series of parallel processes, one of which involves formation and dissipation of 4-hydroxy-2,5,6-trichloroisophthalonitrile (SDS-3701). Chlorothalonil dissipation data were re-analysed to obtain half-life estimates for SDS-3701 soil dissipation. Assuming first order kinetics, non-linear least-squares regression modelling was used to estimate the values of the model parameters. For SDS-3701, half-lives between 6 and 43 days were determined for the various non-sterile soils. An alternative method of data analysis, utilizing a transformation and a linearizing approximation, was also used and gave a similar range of half-lives (Jacobson & Schollenberger, 1992).

The dissipation of chlorothalonil in soils was suppressed by the repeated applications of this fungicide to the soils. The dissipation was due to microbial action, since chlorothalonil disappeared in a nonsterile soil but not in an autoclaved soil. The amendments of the soil with easily decomposable organic materials recovered the suppressed dissipation ability of the soil. The results suggested that easily decomposable organic materials play an important role in the microbial degradation of chlorothalonil in soil (Katayama, et al., 1991).

Fig. 1 lists the structure and identification code of the five soil metabolites that have been identified in aerobic soil studies involving ^{14}C -chlorothalonil in the laboratory. Identifications were based on independent synthesis of authentic standard and GLC or HPLC/MS confirmations. It should be noted that the scheme is a suggested pathway (Frazier, 1993). There is no direct evidence that any of the five soil metabolites are converted

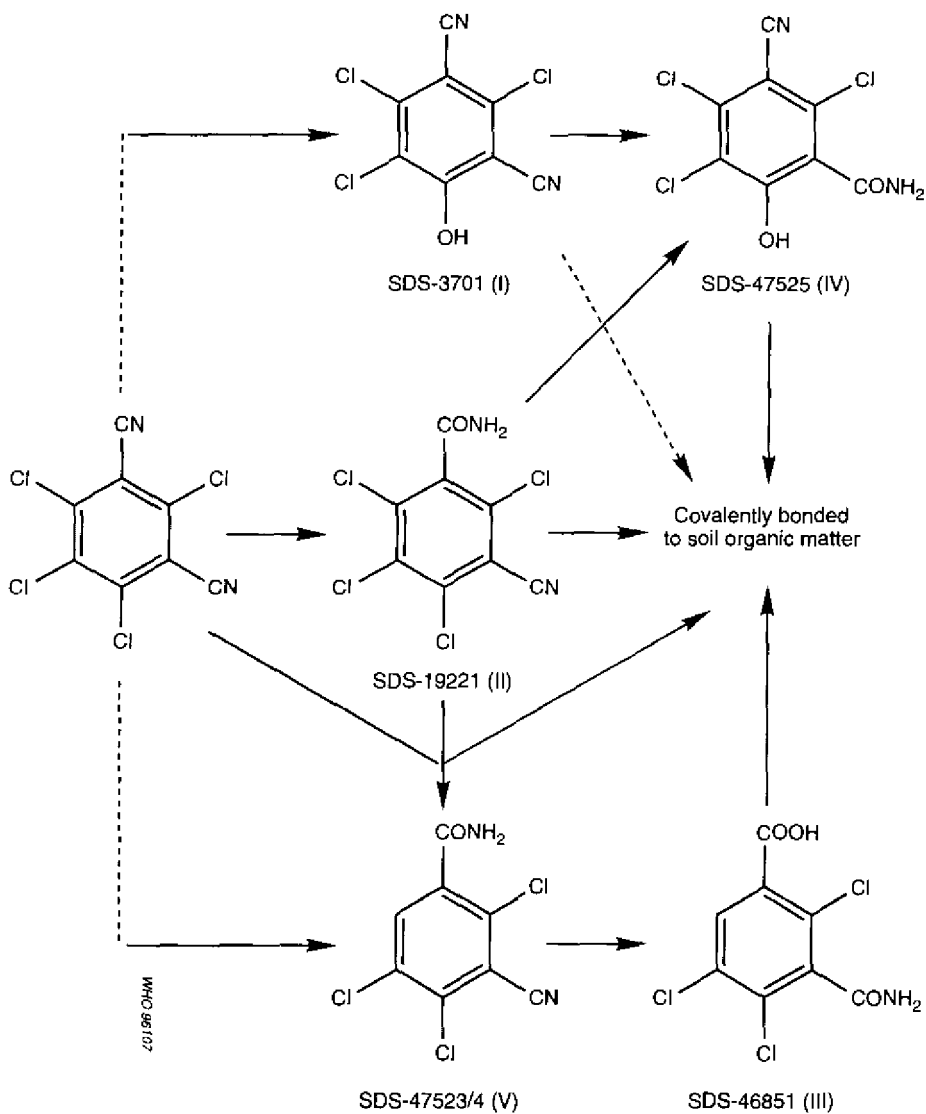


Fig. 1. Suggested pathway for soil degradation of chlorothalonil (from: Frazier, 1993)

directly to "bound" residue. Typical dissipation curves (Figs. 2, 3, 4) show the dissipation of chlorothalonil and the formation/dissipation of the 4-hydroxy-metabolite (SDS-3701); note that the scale for time is not linear. These same dissipation curves show the formation of bound residue. Attempts to liberate and characterize this bound residue have produced limited characterization data and no definitive structure identifications.

A complete picture of all of the known transformations which occur with chlorothalonil under various environmental conditions is given in Fig. 5 (ISK Biosciences, 1995).

On plants, chlorothalonil is metabolized only to a limited extent to the 4-hydroxy metabolite. The majority of the residue remains as the parent compound. Generally less than 5% of the total residue is present as the 4-hydroxy metabolite. A review of plant residues worldwide showed that the 4-hydroxy metabolite level was < 0.1 mg/kg in most of the crops analysed. It accounted for approximately 10% of the total residue in lima beans, 5% in cantaloupes, 2% in peaches and onions, 1% in celery and 0.1% in peanuts (FAO/WHO, 1985). The decline of chlorothalonil residues and the appearance and decline of the 4-hydroxy metabolite on onions is shown in Table 7 (personal communication to the IPCS by the Government of Canada, 1979). The chlorothalonil residue decayed with a half-life of about 3 days.

Table 7. Fate of chlorothalonil on onions after an application at 1.6 kg a.i./ha^a

Days after application	Chlorothalonil (mg/kg)	4-hydroxy metabolite (mg/kg)
0	13.0	0.24
2	7.7	0.15
4	5.8	0.03
6	2.7	0.01
14	0.9	0.005

^a From: personal communication to the IPCS by the Government of Canada (1979).

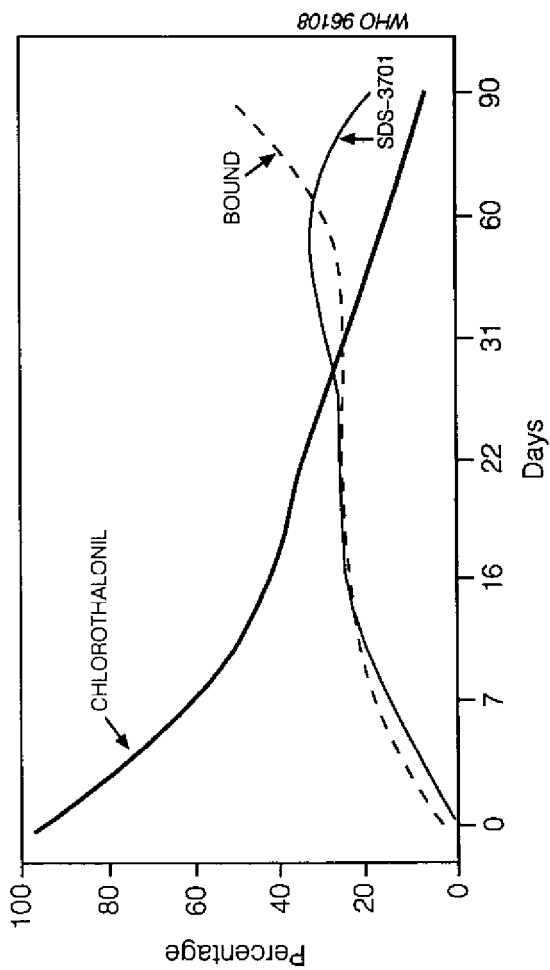


Fig. 2. Degradation of chlorothalonil in peat loam soil (from: Stallard & Szalkowski, 1976)

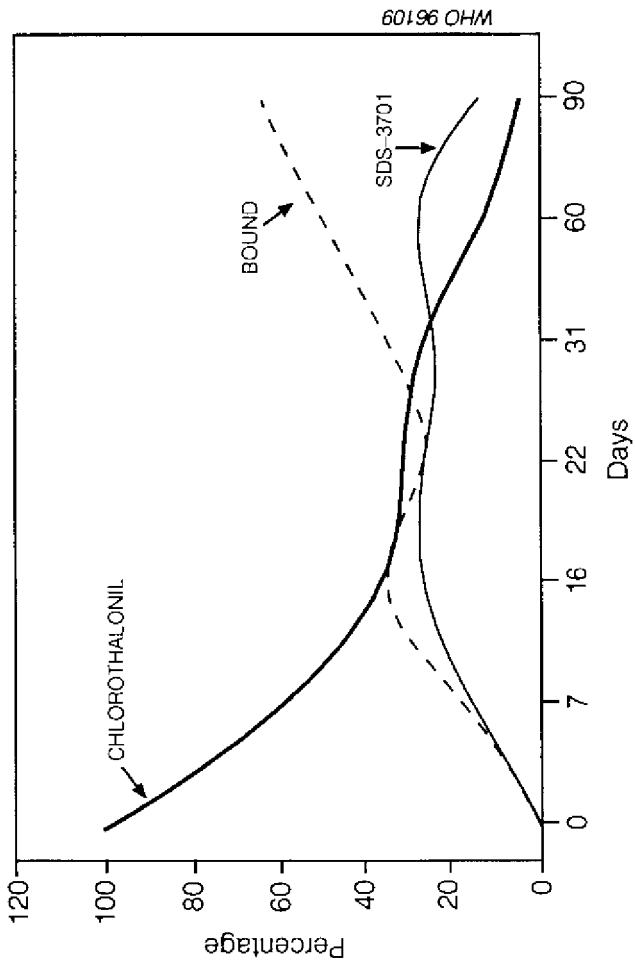


Fig. 3. Degradation of chlorothalonil in sandy loam (from: Stallard & Szalkowski, 1976)

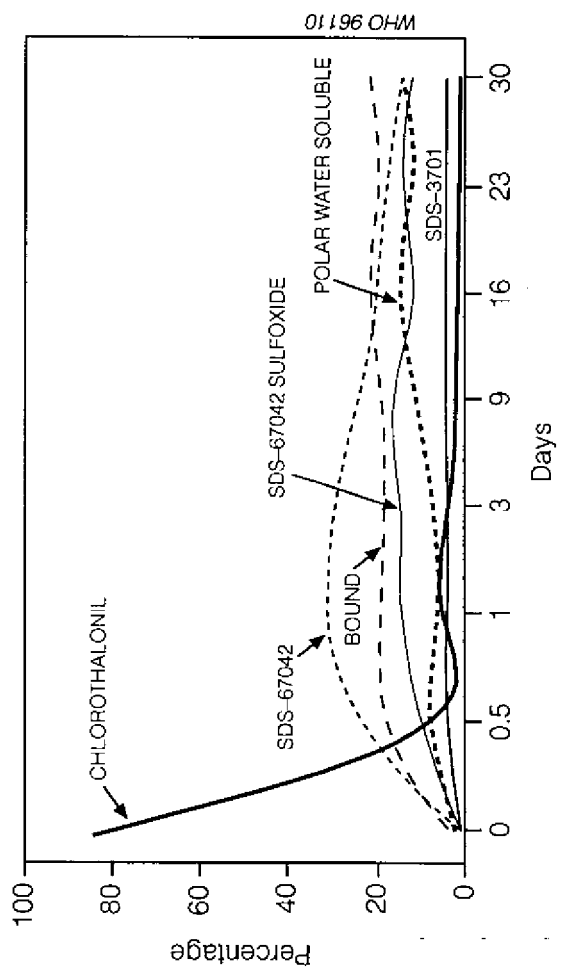


Fig. 4. Degradation of chlorothalonil in aerobic aquatic fresh water/sediment (from: Hatzenbeler, 1991)

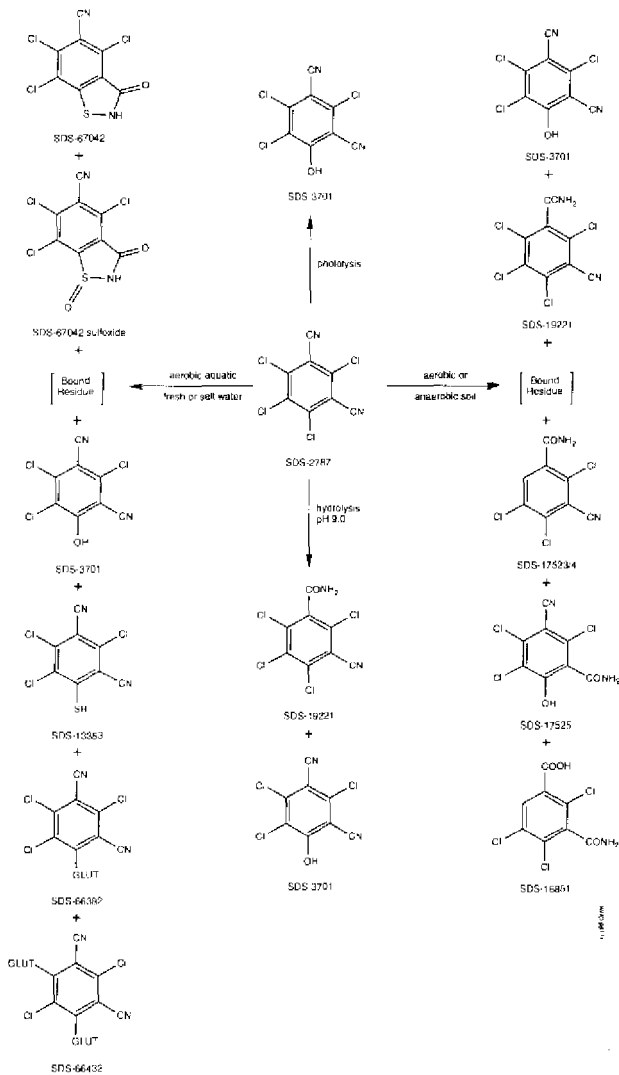


Fig. 5. A summary of chlorothalonil metabolism under various environmental conditions (From: ISK Biosciences, 1995)

Studies with corn silage showed that 90% of chlorothalonil degraded within 18 days (30 to 3 mg/kg). The half-life was approximately 4 days. In a second experiment, the 4-hydroxy metabolite formation was very low in the bound materials (which were converted to an extractable form), representing only about 2% of the chlorothalonil on the first day of ensiling (FAO/WHO, 1978).

After chlorothalonil was applied to growing peanut foliage at 1.26 kg/ha its half-life was 13.6 days (range 7-19 days) under the field conditions of use (Elliott & Spurr, 1993).

In the excretion of ¹⁴C-chlorothalonil metabolites from rainbow trout (*Salmo gairdneri*), the almost complete absence of chlorothalonil itself and the accumulation of ¹⁴C entities in the bile indicated the possibility of glutathione conjugation as the first step in chlorothalonil metabolism (Davies & White, 1985). Further studies showed the existence of mono- and diglutathione conjugates of chlorothalonil in the bile of rainbow trout exposed to ¹⁴C-chlorothalonil (Davies, 1985a).

Studies with liver cytosol from five fish species showed that the enzyme glutathione-S-transferase (GST) is involved in the conversion of chlorothalonil to polar conjugates. Comparisons of GST activity in rainbow trout organs revealed that the potential for chlorothalonil transformation was in the order liver » kidney > spleen, with no activity in bile. Low concentrations of chlorothalonil in water induced fish GST activity for its biotransformation. Hepatic glutathione (GSH) and GST activity for chlorothalonil transformation were compared in three species of fish (*Oncorhynchus mykiss*, *Galaxias maculatus* and *Galaxias auratus*). The order of their asymptotic LC₅₀ values agreed with that of their hepatic GST activities for chlorothalonil transformation and was consistent with a detoxification role for GSH-chlorothalonil conjugation (Davies, 1985b). A study involving co-exposure to zinc and chlorothalonil indicated that metallothionein does not play a significant role in chlorothalonil detoxification in fish at sublethal exposures (Davies, 1985c).

Small amounts of the 4-hydroxy metabolite were found in the milk and kidney of a cow fed 250 mg chlorothalonil/kg in its feed. Only 0.2% of the ingested chlorothalonil was eliminated in the milk as the 4-hydroxy metabolite (Ladd et al., 1971).

4.2.2 *Abiotic degradation*

Chlorothalonil does not break down in aqueous solution (0.5 mg/litre) in the dark at pH 5 or 7. It is hydrolysed at pH 9, over 50% disappearing in 49 days, with the formation of 4-hydroxy-2,5,6-trichloroisophthalonitrile and 3-cyano-2,4,5,6-tetrachloro-benzamide (Szalkowski & Stallard, 1977).

Chlorothalonil degrades very slowly under aqueous photolytic conditions to the 4-hydroxy metabolite. The half-life was found to be approximately 65 days (ISK Biotech proprietary information).

4.2.3 *Bioaccumulation*

In a study of the uptake and elimination of ^{14}C -chlorothalonil in rainbow trout, two groups of fish were exposed to 10 $\mu\text{g}/\text{litre}$ of the compound in flow-through tanks for 96 h (Davies & White, 1985). After exposure was discontinued, the depuration rate was followed for 96 h. There was a very high uptake in the gall bladder and bile (concentration factors up to 4.4×10^5). Uptake was also high in the hind gut, liver, fat and kidney with concentration factors of $2-11 \times 10^3$. After 96 h of exposure, the concentration factor in muscle was 940 and 740, respectively, for the two groups of fish, a level which may give an indication of the magnitude of the whole body bioconcentration factor (BCF) for rainbow trout (not measured).

After transfer to clean water, gall bladder levels dropped rapidly, and so did gill and blood levels. In one group of fish, concentrations in both liver and kidney doubled until 24 h after transfer and thereafter dropped to the levels in the other group. Concentrations in the spleen in both groups continued to increase throughout the depuration period. Muscle levels dropped only slowly and remained around 1 $\mu\text{g}/\text{g}$. The high concentrations found in the gall bladder and bile are consistent with the fact that chlorothalonil is excreted from fish as glutathione conjugates (Davies & White, 1985).

Bluegill sunfish exposed to 8 μg ^{14}C -chlorothalonil/litre in a flow-through system for 30 days showed a plateau of ^{14}C uptake within 14 days. The residues in whole fish at 30 days were 264 times the water concentration. When the fish were placed in clean water, 80% of the radioactive residues were lost within 14 days. Bioaccumulation in catfish, in a static system, showed a 16-fold

concentration at 26 days. In this case 90% of the ¹⁴C residues were depurated in 14 days after removal from the treated water. The 4-hydroxy metabolite did not bioaccumulate in fish (SDS Biotech Corporation, 1972).

In tanks containing stream water with chlorothalonil at 20 µg/litre, uptake of the compound occurred in algal growths attached to bottom pebbles. Analysis of the algal growths showed a concentration factor for chlorothalonil of 270 times after 14 days of static exposure. Since this represented only 9.5% of the initial dose it seems that the removal of chlorothalonil from the water is enhanced by its conversion to polar metabolites in addition to bioconcentration (Davies, 1988).

4.3 Waste disposal

Chlorothalonil can be incinerated in units operating at 850 °C fitted with off-gas scrubbing equipment (Lawless et al., 1975).

The disposal methods for waste pesticides and containers advocated by FAO and GIFAP should be applied to unused chlorothalonil products and their empty packages (FAO, 1985; GIFAP, 1987).

5. ENVIRONMENTAL LEVELS AND HUMAN EXPOSURE

5.1 Environmental levels

5.1.1 Air

Chlorothalonil was detected (amongst other pesticides) in 3 out of 9 outdoor and indoor samples and 1 out of 9 personal monitoring samples in 9 homes in Jacksonville, Florida, USA. No actual figures were reported (Lewis et al., 1988).

Average exposures to chlorothalonil of 173 persons in Jacksonville, Florida and Springfield, Massachusetts, USA were 0.7 ng/m^3 (personal exposure) and 0.5 ng/m^3 (outdoor air concentrations) (Wallace, 1991).

Chlorothalonil was not detected in 51 samples in an Environmental Survey of Chemicals in Japan in 1991 (personal communication by the Office of Health Studies Environment Agency, Tokyo, 1992).

5.1.2 Water

O'Neil (1991) studied concentrations of chlorothalonil in water and sediment following overspraying of a pond (0.2 ha and 0.5 m deep) at 875 g a.i./ha (Ernst, 1991). Water and sediment were monitored in a stream flowing out of the pond at the outlet and 30 m downstream. The stream was approximately 1 m wide and 0.5 m deep and ran at $0.033 \text{ m}^3/\text{sec}$ for the first overspray and at $0.015 \text{ m}^3/\text{sec}$ for the repeat spray. Whole water samples were filtered for separate measurement of chlorothalonil in water and sediment. Following the first spraying, samples were taken at the downstream site at 30 min intervals up to 6 h after spraying and further samples were collected 10 and 24 h after spraying. Initial water concentrations of up to $60 \mu\text{g}/\text{litre}$ fell rapidly to around $15 \mu\text{g}/\text{litre}$ 2 h after spraying. The water concentration was $1.9 \mu\text{g}/\text{litre}$ 10 h after spraying, and at 24 h there was no measurable chlorothalonil. In the second spraying at the lower stream flow rate, whole water samples were taken more frequently over the 2 h following application. Concentrations peaked at $350 \mu\text{g}/\text{litre}$ at the pond outlet and 30 m downstream, respectively, 20 to 30 min after application, falling to between 50 and $100 \mu\text{g}/\text{litre}$ at 2 h. A concentration of $6.3 \mu\text{g}/\text{litre}$ was found 12 h after spraying. Total chlorothalonil mass was measured on

suspended sediment following the first spraying and showed 10 µg persisting for 1.5 h after spraying and thereafter falling to approximately 0.01 µg at 10 and 24 h. The report did not make clear the volume of water filtered, which appears, however, to have been 1 litre. Environmental conditions such as total organic carbon (TOC), pH, temperature and water hardness were not reported; consequently their impacts on degradation could not be evaluated.

Chlorothalonil was detected on occasions at concentrations up to 3.6 µg/litre in a tile drainage system from a farm in Manitoba, Canada, where the fungicide was sprayed routinely. It was detected on one occasion (0.06 µg/litre) in the sump well outflow draining to a municipal ditch (Krawchuk & Webster, 1987).

Over a 5-year period (1986-1990), water was sampled and analysed from 1300 community water systems and rural domestic wells for 101 pesticides, including chlorothalonil. Chlorothalonil was not detected in any of these samples although the reporting limit was 0.12 µg/litre, which represented the minimum quantification limits for this particular pesticide in the study (US EPA, 1990).

Chlorothalonil was not detected in 57 water samples, 30 sediment samples and 30 fish samples in an Environmental Survey of Chemicals in Japan in 1991 (personal communication by the Office of Health Studies, Environment Agency, Tokyo, 1992).

5.1.3 Soil

Levels of chlorothalonil and its metabolite SDS-3701 (see section 4.2.1) in soil were reported after three annual treatments (Kenyon & Ballee, 1990; King et al., 1991, 1992). Four plots were established of bare untreated and treated, winter wheat treated and untreated at two different sites, Osterwede and Rohlstorf (Germany). Treatment consisted of an annual chlorothalonil application of 2.2 kg a.i./ha. Soil samples were taken before and after each treatment. No chlorothalonil was detected in any of the untreated samples. Consistently there was no carry over from one year to another. Levels in soil were highest 2 or 3 days after the treatment (sampling depended on the sites), with mean levels in the bare plots around 0.40 mg/kg and in those with wheat around 0.34 mg/kg (values ranging between 0.07 and 0.64 mg/kg). Between 52 and 60 days after each treatment, levels were 0.02-0.03 mg/kg in plots with wheat while in bare plots they were generally below the detection limit of 0.01 mg/kg. Before each

treatment in the previously treated plots the level of metabolite SDS-3701 ranged from the limit of detection (0.01) to 0.03 mg/kg, which was the same as the level 2 or 3 days after treatment. However, between 52 and 60 days after treatment (depending on the site) levels rose at the Osterwede site to 0.07 mg/kg for the bare treated plot. One year after the last treatment, levels of SDS-3701 ranged from the detection limit to 0.03 mg/kg.

5.1.4 Food crops

Chlorothalonil is used as a broad spectrum fungicide on vegetables, fruit trees, small fruit bushes and other agricultural and horticultural food crops. Its use is intended to protect crops up to harvesting, hence small residues will be present at that time. The residue levels expected in crops at harvest can be derived from the numerous supervised trials that have taken place on many crops in countries all over the world (FAO/WHO, 1975, 1978, 1979, 1980, 1982, 1985a, 1986a, 1990a).

The amount of residue at harvest depends upon factors such as the application rate, time interval between the last application and harvest, and the type of crop. Residues are composed mainly of chlorothalonil, and only negligible amounts of the metabolite 4-hydroxy-2,5,6-trichloroisophthalonitrile(SDS-3701)are present (see Table 7 for example).

The decline of chlorothalonil residues on food crops after application is shown by the field treatment of apples and pears against *Botrytis cynerea* by spraying with a chlorothalonil flowable formulation and then harvesting at intervals after treatment (Camoni et al., 1991). The results are shown in Table 8.

Table 8. Decline of chlorothalonil residues

Days after treatment	Pears (mg/kg)	Apples (mg/kg)
0	3.85	2.35
7	2.48	1.73
14	2.00	0.92
28	1.35	0.98

From: Camoni et al. (1991)

Similar examples of the decline of chlorothalonil residues have been given for grapes in Australia, Germany and South Africa (FAO/WHO, 1985a). The decline of residues in onions is shown in Table 7. The distribution of the residues on this plant showed that the levels in the older outer leaves were about 5 times above those in the younger leaves (2.4 and 0.51 mg/kg, respectively).

Pre-harvest intervals are set on the basis of supervised trials, e.g., 7 days for apricots, and cherries in Australia, 7-14 days for grapes in Australia and 7 days for onions in the Netherlands (FAO/WHO, 1990a).

One of two samples of currants from growers in the United Kingdom had a residue level of 7.5 mg/kg 54 days after the last of three treatments at half the recommended rate of application. The residue level on the second sample was < 0.5 mg/kg 76 days after two applications at the maximum rate (UK, 1985).

5.1.5 Dairy produce

There have been no reports of chlorothalonil residues in dairy produce. However, some indication can be gained from studies on dairy cattle fed high levels of the compound. In one cow fed 250 mg chlorothalonil/kg feed for 44 days, no chlorothalonil was detected in the milk and only 0.2% of the dose appeared as the 4-hydroxy metabolite. Neither compound could be detected in muscle or fat and only a low level of the 4-hydroxy metabolite (0.7 mg/kg) was found in the kidney (Ladd et al., 1971; Wolfe & Stallard, 1971). In another study, groups of four cows were fed chlorothalonil combined with the 4-hydroxy metabolite at levels up to 250 and 0.6 mg/kg, respectively, for 30 days. At the end of the period half the cows were sacrificed and half continued for a 32-day recovery period. No chlorothalonil (< 0.02 mg/kg) was found in milk. Small residues of chlorothalonil and the 4-hydroxy metabolite were detected in muscle, fat, liver and kidney after 30 days administration but none were detected in these organs after the 32-day recovery period (FAO/WHO, 1975). No chlorothalonil (< 0.03 mg/kg) was detected in milk from a cow fed the compound at 5 mg/kg in its rations for 4 days (Gutenmann & Lisk, 1966).

5.1.6 Animal feed

Dry cannery waste (tomato pomace), sometimes used for animal feed, contained < 1 mg/kg chlorothalonil plus its 4-hydroxy metabolite (in the ratio 6:1) as a residue (FAO/WHO, 1978).

5.2 General population exposure

5.2.1 Food

In a study of imported fruit and vegetables in Finland, chlorothalonil levels of 0.02-0.15 mg/kg in strawberries, 0.01-0.86 mg/kg in Chinese lettuce and 0.12-1.2 mg/kg in peaches were found (personal communication to the IPCS by the Government of Finland, 1979).

No chlorothalonil (< 0.01 mg/kg) was detected in a US Food and Drug Administration (FDA) total diet study in the USA in 1976 or 1977 (personal communication to the IPCS by J.R. Wessel, 1979). In a Canadian total diet survey, chlorothalonil was detected in one out of six composite samples of garden fruits at the detection level (0.02 mg/kg). On the basis of this one sample, a dietary intake of 0.04 µg per person per day was estimated (McLeod et al., 1980).

Chlorothalonil was detected (0.001-1.35 mg/kg) in most samples of apples, peaches and other fruit and vegetables marketed in Tokyo (Koseki et al., 1980).

No chlorothalonil (< 0.005 mg/kg) was detected in samples of potatoes in Sweden in 1979. During 1981-1983, 1070 out of 1085 samples of domestic and imported commodities in Sweden had chlorothalonil residues below 0.21 mg/kg. Samples having higher residues included one of cauliflowers (out of 165) at 0.41 mg/kg, one of cucumbers (out of 580) at 0.23 mg/kg and two of strawberries (out of 143) at 2.9 mg/kg (personal communication: data submitted to the IPCS by the Government of Sweden and entitled "Chlorothalonil residues in imported and domestic commodities - 1981 to 1983").

In 1982, analysis at the point of retail in the United Kingdom showed chlorothalonil residues below 0.5 mg/kg in 41 samples of strawberries, 15 of gooseberries, 13 of currants and 9 of berries. Other analyses during 1981-3 showed that only one out of 30 samples of imported strawberries, 2 out of 15 samples of domestic celery and 5 out of 40 of gooseberries had chlorothalonil residues above 0.1 mg/kg (UK, 1985).

In the United Kingdom, chlorothalonil residues in bananas (imported), chinese cabbage (all origins) and parsnips (United Kingdom origin) were below the reporting levels of 0.2, 1.0 and

0.01 mg/kg, respectively, in 1988-1989. During the same period, one sample out of ten of imported strawberries contained 0.1 mg/kg (UK MAFF & HSE, 1990).

Residues of chlorothalonil in foodstuffs are decreased by processes such as washing. For example, it was shown that 94% of the residue could be removed by washing tomatoes and that there was no detectable residue in canned tomato pulp, paste or juice. Peaches washed in water followed by a caustic rinse showed a 97% removal of field residues. No chlorothalonil was detected in canned peach puree (FAO/WHO, 1978).

In a Honduran study, unwashed bananas had a maximum residue level of 0.17 mg/kg and a mean of 0.08 mg/kg. This was reduced to 0.02 mg/kg after washing. No chlorothalonil was found in the edible pulp (< 0.01 mg/kg). Similar results were obtained in the Philippines (FAO/WHO, 1980).

Trimming and peeling also removes a large proportion of residues from some foodstuffs. For example there are significant reductions after trimming the outer leaves from cabbages and lettuces. Most of the residue is removed when cucumbers, melons, peanuts and potatoes are peeled (Diamond Shamrock, 1974).

As much as 85-98% of chlorothalonil added to tomatoes or green beans was lost during cooking in open vessels. Only 2.4% was converted to the 4-hydroxy metabolite, which was stable to cooking (SDS Biotech Corporation, 1983a).

5.3 Occupational exposure

The exposure of a tractor driver applying chlorothalonil to ornamental plants in Florida, USA, was assessed. Total-body exposure rates, estimated from external exposure pads and air sampling, were low (approximately 5 mg a.i./h) (Stamper et al., 1989a). In the case of a greenhouse drencher, this exposure was approximately 100 mg a.i./h (Stamper et al., 1989b).

Occupational exposure to four insecticides and two fungicides was measured for 151 commercial tree and shrub applicators in the USA who used hand-held equipment when spraying pesticides. The study was conducted for 3 consecutive years: 1985, 1986 and 1987. Worker exposure was determined by collecting full-shift, breathing zone air samples. Sampling was conducted with battery-operated constant-flow air-sampling devices. Chlorothalonil was

detected in only one out of 14 samples at 0.011 mg/m³ (Leonard & Yeary, 1990).

Spencer et al. (1991) estimated the dermal exposure of workers on mechanical tomato harvesters to residues of chlorothalonil. An average of 499.6 µg/h was obtained by gauze pad dosimeters placed outside the workers' clothing, whereas 43.4 µg/h was obtained by undershirt dosimetry. The results showed that regular work clothing provides an excellent protection (90% reduction in dermal exposure) against chlorothalonil. Air concentrations in the field were also determined and averaged 0.002 to 0.02 µg/litre, which contributed 8 to 28% to the total exposure.

The exposure of 11 pesticide operators mixing, loading or applying chlorothalonil fungicide formulations by aerial or ground applicators has been assessed. The highest exposure was on the hands (1.7 mg/m² per h) (Diamond Shamrock, 1980).

6. KINETICS AND METABOLISM IN LABORATORY ANIMALS AND HUMANS

6.1 Absorption

Biliary excretion of radioactivity was studied in groups of six male Sprague-Dawley rats administered a single oral dose of 1.5, 5, 50 or 200 mg/kg body weight ^{14}C -chlorothalonil (98% radiochemically pure), uniformly labelled in the aromatic ring, as a suspension with a mean particle size of 3.6 to 5.0 μm in 0.75% methylcellulose in water. The bile duct was cannulated and bile was collected in 1-h fractions for 48 h after dosing. Blood, urine and faeces were also collected at various times after dosing and at termination. During the 48 h after a single dose of 1.5, 5, 50 and 200 mg/kg body weight, biliary excretion was 22.5, 16.4, 16.3, and 7.7% of the administered dose, respectively. Profiles of radioactivity excretion after the two low doses were quantitatively different from those obtained after the two high doses. The authors interpreted these results as indicative of a change in metabolism occurring between 5 and 50 mg/kg body weight, possibly due to saturation of biliary excretion. Mean urinary excretion during the 48 h after dosing was 8.0, 8.2 and 7.6% of the administered dose at 1.5, 5 and 50 mg/kg body weight, respectively, and only 4.7% at the high dose level of 200 mg/kg body weight. Excretion of radioactivity in the urine within 6 h after dosing was inversely related to the dose administered. Total recovery of radioactivity in this study was 89-99% in the three low-dose groups and 74% in the high-dose group. After doses of 1.5, 5, 50 and 200 mg/kg body weight, rats absorbed 32, 25.7, 25.9 and 15.5% of the administered dose, respectively. It was concluded by the authors that enterohepatic circulation or reabsorption of biliary metabolites from the gastrointestinal tract did not contribute significantly to the amount of radiolabel in the kidney. Based on a one-compartment model for chlorothalonil absorption and excretion and using several assumptions, it was calculated that the rate of absorption of the 200 mg/kg body weight dose was only twice as fast as that of the 50 mg/kg body weight dose (Marciniszyn et al., 1986).

In a study by Chin et al. (1981), absorption was compared by the oral, dermal and endotracheal routes with a 1 mg/kg body weight dose of ^{14}C -chlorothalonil in male Sprague-Dawley rats. The comparisons were made on the basis of blood levels and urine excretion. In each case, absorption was highest by endotracheal

administration and lowest by the dermal route. Less than 6% of the administered dose was recovered in blood and urine within 48 h after dosing.

When ^{14}C -chlorothalonil was introduced into sacs formed from the upper section of rat small intestine, no unchanged substance passed through the mucosa and was transferred to the serosal side of the sac. These data suggest that chlorothalonil is very rapidly conjugated, since *in vivo* studies have not identified chlorothalonil itself in body fluids or tissues after oral administration to rats (Savides et al., 1986e).

^{14}C -chlorothalonil was applied to the skin of male rats at an averaged dose of 1167 $\mu\text{g}/\text{rat}$ (= 5 mg/kg) on an area of 25 cm^2 . The amount absorbed was deduced from the amount remaining on the treated skin area and the amount of radioactivity found at each time interval in urine, faeces and carcass. Approximately 28% of the applied dose was absorbed over the experimental exposure period of 120 h. The absorption appeared to be time-dependent, about 6.3% of the applied dose being absorbed during each 24 h period. Radioactivity appeared quickly in blood and rose steadily up to 72 h, when it reached a plateau (Marciniszyn et al., 1984a).

In a study by Magee et al. (1990), four monkeys were treated dermally with 5 mg/kg body weight of ^{14}C -chlorothalonil under a non-occlusive patch. After 48 h the patch was taken off and the skin was washed. About 90% of the dose was recovered from the surface and about 2.26% was completely absorbed through the skin. The urine contained 1% of the dose, but methylated mono-, di- and trithiols were not detectable in the urine.

6.2 Distribution

Groups of male and female rats were administered ^{14}C -chlorothalonil orally, in microparticulate suspension, as single doses of 5, 50 or 200 mg/kg, and tissue activity was determined after 2, 9, 24, 96 and 168 h (Marciniszyn et al., 1984b, 1985a). With the exception of gastrointestinal tract tissues the greatest concentration of radioactivity was found in the kidneys, at each dose level, followed by those in liver and whole blood. The peak concentrations in kidney occurred at 2 h after 5 mg/kg, 9 h after 50 mg/kg and 24 h after 200 mg/kg. Similar shifts in peak time with dose occurred in the liver and blood. In terms of the original dose, kidneys, liver and blood each contained 0.7% of the label 2 h after 5 mg/kg and 0.3% (kidney), 0.14% (liver) and 0.23% (blood) after 24 h in female rats.

Distribution of radioactivity was also studied after repeated oral administration of ^{14}C -chlorothalonil to male rats. Five doses were given at 24 h intervals at concentrations of 1.5, 5, 50 or 160 mg/kg. The rats were killed 2, 9, 24, 96 and 168 h after the last dose. The distribution of activity showed a similar profile to that after single dosing, i.e. the highest concentrations occurred in kidneys, followed by liver and blood, at all doses and times. At all dose levels, the concentrations peaked 2 h after the last dose. The percentage of the dose found in the kidney at this time was 0.28% and 0.20% at the 1.5 and 5 mg/kg dose levels, which was significantly higher than that found at the higher doses (about 0.09%). At dose levels up to 50 mg/kg there was significant depletion of radioactivity from the blood during the 24 h between doses. In the kidney there was a trend to slower overall depletion with increase in dose (Savides et al., 1986a).

A study in mice showed that the distribution of activity in non-gastrointestinal tract tissues was similar to that in rats after a single oral dose of ^{14}C -chlorothalonil. The kidney had the highest concentration of radioactivity after doses of 1.5, 15 or 105 mg/kg (Ribovich et al., 1983).

6.3 Metabolic transformation

6.3.1 Rat

Male Sprague-Dawley rats were administered, via oral gavage, ^{14}C -chlorothalonil (purity 99.7%) at a dose level of 200 mg/kg in order to isolate and identify the urinary metabolites. Urine was collected 17, 24 and 48 h after dosing. Urinary metabolites accounted for 2.4% of the administered dose and, except for 30% of the radiolabel which was non-extractable from the urine, were found to be trimethylthiomonochloro-isophthalonitrile and dimethylthiodichloro-isophthalonitrile. These thiols were excreted in urine both as free thiols and as their methylated derivatives. The authors suggested a metabolic pathway such that hepatic metabolism proceeds through conjugation with GSH followed by enzymatic degradation. The smaller conjugates are then transported via the bloodstream to the kidney, where they are converted to thiol metabolites and excreted in the urine (Marciniszyn et al., 1985b).

A study was also carried out in rats given five daily oral doses of ^{14}C -chlorothalonil (1.5, 5, 50 or 160 mg/kg per day). Urine samples, acidified and extracted with ethyl acetate, showed

decreasing extractability of radioactivity with increasing dose. GC/MS analysis identified methylated or partly methylated dithiol and trithiol derivatives of chlorothalonil from the first dose onwards. The percentage of the trithiol derivative excreted was constant with increasing dose while the dithiol increased with dose. Multiple dosing resulted in a decreasing daily excretion of total thiol derivatives. These results emphasize the probable involvement of glutathione in the metabolic pathway for chlorothalonil (Savides et al., 1986b).

A group of three rats, pretreated with the γ -glutamyl transpeptidase inhibitor AT-125, were dosed with 50 mg/kg ^{14}C -chlorothalonil, while three other rats were given chlorothalonil only. Urine samples were acidified and extracted with ethyl acetate. The group of rats pre-treated with AT-125 showed only 15% of radioactivity extractable after 12 h, while the other group showed 75% extractability. The non-extractable fraction from the inhibitor-treated rats contained glutathione conjugates of chlorothalonil. The kidneys contained 2-3 times more radioactivity than those of the untreated rats. These results gave further support to the hypothesis that glutathione is involved with chlorothalonil metabolism (Marciniszyn et al., 1988).

The production of metabolites was also studied in groups of rats following dermal administration. ^{14}C -chlorothalonil (4.6 mg/kg) was applied to a shaved area of the dorsal region. The area was covered and exposure continued for 48 h. Urine samples collected at 24 and 48 h were acidified and extracted with ethyl acetate. The extracts were submitted to reverse-phase HPLC/LSC followed by methylation and further clean-up. The trithiol derivative of chlorothalonil was the major metabolite in all samples. The excretion of total thiol metabolites was at least 20-fold less than that resulting from oral dosing at the same dose level (Savides et al., 1987a).

The radiolabelled monoglutathione derivative of chlorothalonil was administered to male rats (115 mg/kg) as a single oral or intraperitoneal dose. Six hours after intraperitoneal dosing the blood level was 10 times higher than after oral dosing. The proportion of the administered intraperitoneal dose in the kidney was 16 times higher than after oral dosing. Urine from the orally dosed rats contained 9% trithiol derivative and 5% dithiol, while intraperitoneally dosed rats showed < 1% dithiol derivative and none of the trithiol in urine. This indicates that the orally administered monoglutathione conjugate is further conjugated

with glutathione in the gastrointestinal tract prior to absorption (Savides et al., 1986f).

Nine germ-free male rats each received approximately 56 μCi ^{14}C -chlorothalonil in a single oral dose of 50 mg/kg. Urine and faeces were collected over a 96-h period, and the urine was processed to identify and quantify thiol derivatives of chlorothalonil. These derivatives were detected in only three of the nine rats and represented < 0.03% of the dose. This is fifty times less than that obtained for normal rats. There is therefore strong evidence that intestinal microflora make a significant contribution to the metabolism of chlorothalonil after oral administration in the rat (Savides et al., 1990a).

The HPLC analysis of faecal extracts from rats dosed with 200 mg chlorothalonil/kg showed that 28% was excreted unchanged and 5% was converted to 4-hydroxy 2,3,5-trichloroisophthalonitrile. The amounts after a dose of 5 mg/kg were 1.6 and 6.2%, respectively (Ignatoski et al., 1983).

The HPLC analysis of faeces from rats given ^{14}C -chlorothalonil orally at 5, 50 and 200 mg/kg showed the presence of at least seven radioactive components. Two of the peaks had the same retention times as chlorothalonil and its 4-hydroxy metabolite. A higher proportion of the metabolite was present after the 5 mg/kg dose than after the higher doses. The majority of the activity was unextractable and was therefore bound to faecal components (Lee et al., 1982).

6.3.2 Dog

Male beagle dogs were given ^{14}C -chlorothalonil at a dose level of 50 mg/kg either by gelatin capsule or by gavage. In each case the urinary excretion of radioactivity was very small and none of the methylated thiol derivatives of chlorothalonil were detected (Savides et al., 1989, 1990b).

6.3.3 Monkey

Four male Chinese rhesus monkeys were dosed with ^{14}C -chlorothalonil by gavage at 50 mg/kg body weight suspended in 0.75% aqueous methylcellulose. Extraction of urine, collected over 48 h, with acidified ethyl acetate showed that 32-65% of the radioactivity was extractable. The total amount of chlorothalonil thiol derivatives excreted was 0.001-0.01% of the administered

dose, mainly as the trimethylthiol entity. This was more than 100 times less than that excreted from the rat (Savides et al., 1990c).

6.4 Elimination and excretion

6.4.1 Rat

The main route of elimination of chlorothalonil from the rat after oral dosing is via the faeces. The percentage eliminated was consistent for males and females, at doses of 1.5-200 mg/kg and from single or repeated doses. The amount eliminated was consistently above 82%, the majority appearing in the first 48 h at low doses and within 72 h at high doses (Marciniszyn et al., 1984b, 1985a).

Biliary excretion at dose levels up to 5 mg/kg is rapid, peaking at 2 h, and is more prolonged at levels of 50 mg/kg or more. Excretion decreases with increasing dose, from 22.5% at 1.5 mg/kg to 7.7% at 200 mg/kg over 48 h. Studies using bile duct cannulation indicate that the excretion is saturated at 50 mg/kg or more. Comparison with non-cannulated rats showed that there was no difference in the radioactive concentration found in the kidney, indicating that enterohepatic circulation of biliary metabolites did not play a significant role (Savides et al., 1986c).

The fate of orally administered ¹⁴C-chlorothalonil (purity 99.7%) at three dose levels (5, 50 and 200 mg/kg) was investigated in Sprague-Dawley rats to determine the effects of increasing doses of the test material. Four animals of each sex at each dose level were killed 2, 9, 24, 96 and 168 h after dosing and urine, faeces and selected tissues were assayed for radioactivity. The average recovery of the radiolabel at each of the dose levels was approximately 89% for males and 96% for females. The major route of elimination was via the faeces (83-87%) and was essentially complete by 48 h in low-dose females and low/mid-dose males, and by 72 h in the mid/high-dose females and high-dose males. A delay in stomach-emptying time was observed for mid- and high-dose males and females. Urinary excretion was 92-93% complete for low-dose rats within 24 h, mid-dose rats within 48 h, and 95% complete for high-dose rats within 72 h. Urinary excretion of the radiolabel at the three dose levels was 5-7% of the administered dose in males and 5-11.5% in females. Urinary excretion was essentially saturated as the dose level increased. The highest concentrations of radiolabelled material in non-gastrointestinal tissues were found in the kidney, being

approximately 0.7% of the dose per gram of kidney for males and 0.4% in females at peak concentration (2 h) for the 5 mg/kg dose level. Kidney concentrations were greatest at 2, 9 and 24 h for the low, mid and high doses, respectively (Marciniszyn et al., 1984b, 1985a).

When ¹⁴C-chlorothalonil was applied dermally to male rats at 5 mg/kg the major route of excretion was the faeces. Approximately 18% of the dose was excreted by this route in 120 h compared to about 6% via urine (Marciniszyn et al., 1984a).

After administration of 1 mg chlorothalonil/kg by the oral, dermal or endotracheal routes, the excretion in urine during 24 h was 2.9, 0.9 and 5.7%, respectively (Chin et al., 1981).

A study involving intubation of the 4-hydroxy metabolite of chlorothalonil to rats at 4 or 43 mg/kg showed that the majority of the dose was excreted in the faeces and a small amount in the urine (Jarrett et al., 1978).

6.4.2 Mouse

In male mice, dosed orally with 1.5, 15 or 105 mg ¹⁴C-chlorothalonil/kg, the major route of elimination was via the faeces. This was complete by 24 h for the two lower doses and by 96 h for the highest dose. Urinary excretion at all doses varied between 5 and 10% of the administered dose (Ribovich et al., 1983).

6.4.3 Dog

Most of an oral dose of chlorothalonil was excreted in the faeces of beagle dogs. Over 12 days, 99.6% was excreted from two dogs given 50 mg ¹⁴C-chlorothalonil/kg by gelatin capsule, and 76-98% was excreted over 24 h when three dogs were given the same dose by gavage. In both studies the amount excreted in urine was very small, and this occurred mostly within the first 10 h (Savides et al., 1989, 1990b).

6.4.4 Monkey

Oral administration of ¹⁴C-chlorothalonil to four male Chinese rhesus monkeys showed faecal elimination to be the main route of excretion, 52-92% of the dose (50 mg/kg) being excreted in 96 h. Urinary excretion amounted to 1.8-4.1% of the dose. Most of the radiolabel was eliminated in the first 48 h (Savides et al., 1990c).

6.5 Reaction with body components

Incubation of ^{14}C -chlorothalonil with glutathione in aqueous medium in the presence or absence of glutathione-*S*-transferase resulted in the rapid disappearance of chlorothalonil and the appearance of more polar compounds. These were identified as conjugates of chlorothalonil with glutathione, their formation following a step-wise process, i.e., from mono→di→triglutathione conjugates (Savides et al., 1985). This reaction with glutathione parallels similar findings with chlorothalonil in other biological systems such as *Saccharomyces pastorianus* (Tillman et al., 1973).

The incubation of chlorothalonil with rat stomach or intestinal mucosal cells indicated that polar metabolites were formed which were chromatographically similar to glutathione conjugates of chlorothalonil (Savides et al., 1986d).

The *in vivo* action of chlorothalonil on glutathione (GSH) was shown in a rat study where chlorothalonil was dosed orally at 5000 mg/kg. Hepatic GSH was decreased by 40% 18 h later but recovered to a normal value after 48 h. Kidney GSH increased to two times its control level after 48 h (Sadler et al., 1985).

Chlorothalonil has been shown to bind to calf thymus histones, the rate and amount depending upon pH and type of histones. There was little binding to DNA. Treatment of rat liver nuclei indicated similar binding patterns to those for histones (Rosanoff & Siegel, 1981).

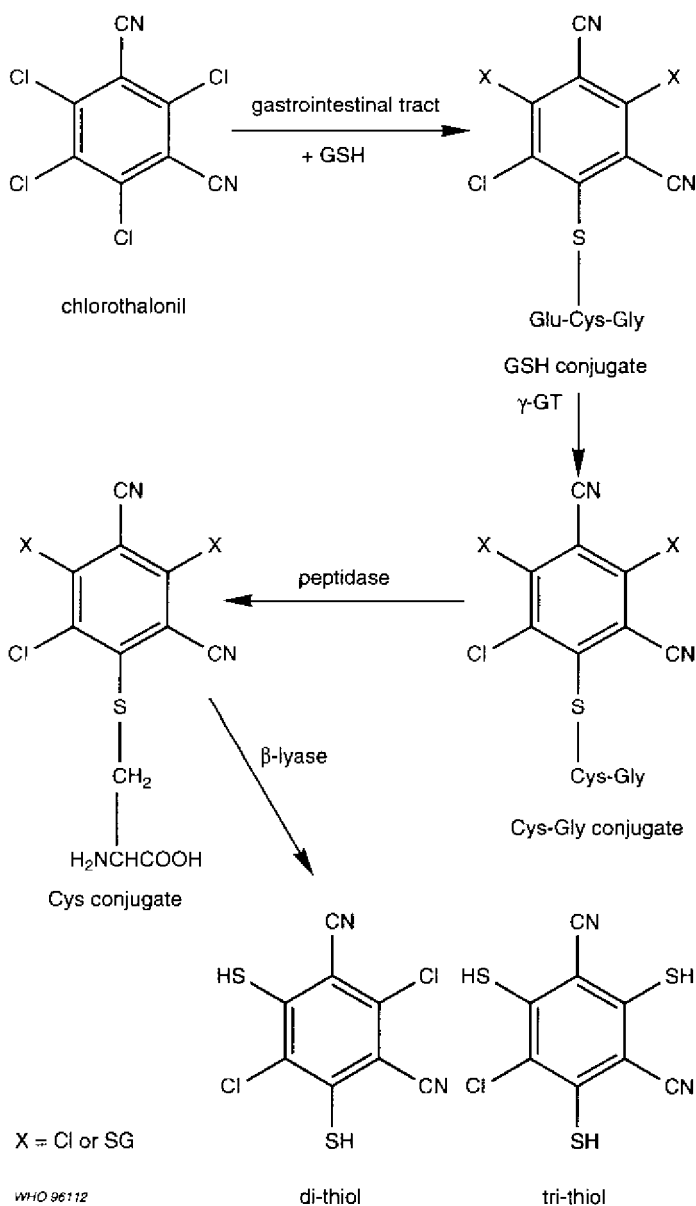
Groups of four rats were administered 50 mg ^{14}C -chlorothalonil per kg orally, killed after 6 h and their kidneys excised. The kidney tissue was homogenized, and protein and DNA were isolated. Radiolabel was found to be bound to kidney protein but not to DNA (Savides et al., 1987b). Kidney tissue from rats dosed 50 mg ^{14}C -chlorothalonil/kg orally was separated by ultracentrifugation into subcellular organelles. The kidneys contained about 0.38% of the original dose, the majority of this activity (81%) being in the soluble fraction. About 10% of the remaining activity was contained in the mitochondrial subfractions (Savides et al., 1987c).

Studies with rat liver and kidney mitochondrial preparations showed that the dithiol derivative of chlorothalonil completely inhibited state 3 mitochondrial respiration. The monothiol derivative affected oxygen uptake by liver mitochondria but not

by kidney mitochondria. The mono- and diglutathione conjugates of chlorothalonil did not affect oxygen uptake by mitochondria. Since cleavage of the glutathione conjugates to give the thiol derivatives takes place in the kidney, this may explain the toxic action of chlorothalonil in this organ (Savides et al., 1988; see also section 7.8).

Available evidence indicates that the enzyme β -lyase is required for the formation of thiol derivatives from cysteine conjugates. The activity of this enzyme has been assessed in rat, mouse and human kidney cytosolic fractions using the perchloroethylene metabolite *S*-(1,2,2-trichlorovinyl)-*L*-cysteine as substrate. The activity of renal β -lyase in human kidney was comparable to that of mouse, but was an order of magnitude lower than that in the rat (Green et al., 1990).

The proposed metabolic pathway for the production of thiol derivatives from chlorothalonil in the kidney is shown in Fig. 6.



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Fig. 6. Proposed pathway for chlorothalonil transformation to thiol derivatives in rat kidney

7. EFFECTS ON LABORATORY MAMMALS AND *IN VITRO* TEST SYSTEMS

7.1 Single exposure

The acute toxicity of chlorothalonil by oral, dermal and inhalation routes of administration is shown in Table 9. Signs of poisoning include depression, diarrhoea and unkempt appearance. An oral LD₅₀ could not be attained in the dog because of emesis. Chlorothalonil administered orally (1 g/kg) to mice increased intestinal motility. The laxative action was reduced by pre-treatment with corn oil (Teeters, 1966).

Table 9. The acute toxicity of chlorothalonil

Species	LD ₅₀ (mg/kg)	Route	Reference
Rat	> 10 000	oral	Powers (1965)
Dog	> 5000	oral	Paynter (1965a)
Mouse	6000	oral	Yoshikawa & Kawai (1966)
Rat (4 hydroxy metabolite)	332	oral	Wazeter (1971)
Rabbit	> 10 000	dermal	Doyle & Elsea (1963)
Rat (f & m) ^a	0.22 mg/litre	inhalation	Danks & Fowler (1988)
Rat (f & m) ^b	0.52 mg/litre	inhalation	Shults et al. (1991)
Rat (f & m) ^c	0.10 mg/litre	inhalation	Shults et al. (1993)

^a LC₅₀ actual concentration

^b Hammermilled technical chlorothalonil - 1 h exposure

^c Hammermilled technical chlorothalonil - 4 h exposure

The acute oral toxicity of the metabolite 4-hydroxy-2,5,6-trichloroisophthalonitrile is greater than that of chlorothalonil

itself, the acute oral LD₅₀ values being 332 and 10 000 mg/kg, respectively).

7.2 Short-term exposure

7.2.1 Oral

7.2.1.1 Rat

When groups of 35 male and 35 female rats were fed chlorothalonil in the diet at dose levels of 0, 250, 500, 750 or 1500 mg/kg diet for 22 weeks, growth was slightly reduced at all levels in males and in the highest two levels in females. Liver and kidney weight was increased in the two highest dose groups in males. Kidney changes, more evident in males than females, occurred at all dose levels and included irregular swelling of the tubular epithelium, epithelial degeneration and tubular dilation (Blackmore & Shott, 1968). In a separate study, no compound-related effects were seen in the kidneys of rats fed chlorothalonil at seven dietary levels from 1-120 mg/kg diet for 17 weeks (Busey, 1975).

When groups of 20 male and 20 female rats were fed chlorothalonil in the diet (0, 40, 80, 175, 375 and 1500 mg/kg body weight per day) for 90 days, a significant dose-related reduction in body weight gain was seen at levels of 375 mg/kg per day or more which was allied to cathartic action at the two highest doses. Male urine output decreased and specific gravity increased at the two highest dose levels. Relative kidney weight was increased in both sexes at all dose levels. A histopathological re-evaluation of the kidneys showed the presence of epithelial hyperplasia of the proximal convoluted tubules at all levels in males and at levels of 175 mg/kg per day or more in females (Wilson, 1981; Wilson et al., 1985c).

Groups of 25 male and 25 female rats were fed chlorothalonil for 13 weeks at 0, 1.5, 3, 10 and 40 mg/kg body weight per day. Five rats of each sex per group were killed at 6 weeks, ten at 13 weeks and the survivors were killed after a further 13-week recovery period. There was no effect on mortality, clinical condition, body weight, food and water consumption, haematology or urinalysis. Increases in kidney (> 3 mg/kg per day) and liver (highest dose) weights and the incidence of hyperplasia and hyperkeratosis of the forestomach squamous epithelium (at 10 and 40 mg/kg per day) returned to normal when treatment ceased.

Histopathological re-examination of the kidney revealed (in males at the highest dose level) an increased incidence of hyperplasia of the epithelium of the proximal tubules at 6 and 13 weeks and after a 13-week recovery period. In a few animals this resulted in an increase in the size of the tubules. An overall no-observed-effect level of 3 mg/kg body weight per day was established, based upon the lack of lesions in the squamous epithelium of the forestomach (Wilson et al., 1983a, 1985a).

The kidney and stomach changes were investigated further in a study where 90 male rats were fed chlorothalonil (175 mg/kg body weight per day) for up to 91 days. Groups of ten rats were killed after 4 or 7 days and 2, 4, 6, 8, 10 or 12 weeks, the kidney and stomach taken for histopathological examination. Groups of ten rats at each interval were taken from a control group and examined. The forestomach effects of chlorothalonil were characterized initially as multifocal ulceration and erosion of the mucosa, developing to squamous epithelial hyperplasia and hyperkeratosis. Within the first week there was vacuolar degeneration, cell death, karyomegaly and regeneration of the proximal tubular epithelium. There was apparent recovery at day 14 with few lesions present in treated animals. Continued administration led to tubular epithelial vascularization, regeneration, hyperplasia and hypertrophy (Ford et al., 1987a).

Chlorothalonil or the monogluthathione conjugate of chlorothalonil was administered in the diet to male Fischer-344 rats in a 90-day study. A third group of rats received only the vehicle (0.5% methylcellulose). The oral administration of approximately equimolar doses of chlorothalonil (75 mg/kg per day) or the monogluthathione conjugate of chlorothalonil (150 mg/kg per day) resulted in significantly increased kidney weight and in treatment-related histopathological changes in the kidney. The primary changes observed were morphologically similar for both compounds and were characterized by proximal tubular hyperplasia, tubular dilation (hypertrophy), vacuolar degeneration and interstitial fibrosis. The oral administration of chlorothalonil resulted in gross and microscopic changes in the non-glandular portion of the forestomach. The oral administration of the monogluthathione conjugate of chlorothalonil did not produce alterations in the rat forestomach. The gross and microscopic changes observed in the forestomach of animals given chlorothalonil were considered to be due to the local irritational effects of chlorothalonil. The alteration of the molecule by the addition of a single glutathione residue appeared to eliminate the

irritation to the forestomach. Thiol metabolites of chlorothalonil were detected in the urine of animals given either the monogluthathione conjugate of chlorothalonil or chlorothalonil itself. The presence of the thiol metabolites, coupled with the similar histopathological changes in the kidney, suggests a common metabolic pathway. The data support the conclusion that the nephrotoxicity produced by chlorothalonil is associated with conjugation with glutathione (Ford et al., 1987b; Wilson et al., 1990).

7.2.1.2 Mouse

Groups of 15 male and 15 female CD-1 mice were administered chlorothalonil in diets at levels of 0, 7.5, 15, 50, 275 or 750 mg/kg diet for 13 weeks. Five animals per group were killed at 6 weeks. There was no effect on clinical condition, mortality, body weight gain or food consumption. Kidney weight was increased in females at 275 and 750 mg/kg. Microscopic re-examination revealed hyperplasia of the epithelium of the proximal convoluted tubules, minimal or slight in severity, in only 4 out of 15 males. This was not considered to be a clear treatment-related effect. There was an increased incidence of hyperplasia and hyperkeratosis of the squamous epithelial cells of the stomach in males and females at levels of 50 mg/kg or more. Mucosal ulceration and submucosal inflammation were observed in some treated animals. The no-observed-effect level was considered to be 15 mg/kg (3 mg/kg body weight per day) (Shults et al., 1983; Busey, 1985).

7.2.1.3 Dog

In a 30-day feeding study, encapsulated chlorothalonil (97.9% purity) was administered daily to groups of two male and two female beagle dogs at 0, 50, 150, or 500 mg/kg body weight per day. The following tissues were examined macroscopically and microscopically at necropsy: brain, liver, kidneys, testes with epididymis, ovaries, adrenals, heart, thyroid, and parathyroid. During treatment the high-dose dogs exhibited emesis and weight loss and reduced food consumption (males only). Female dogs had slightly reduced body weight gains at all doses. At necropsy, the liver weights of high-dose females were slightly increased. There were no microscopic changes in the tissues examined. Due to the reduced body-weight gains of treated females, a no-observed-adverse-effect level (NOAEL) was not established in this study (Fullmore & Laveglia, 1992; FAO/WHO, 1993b).

In a 16-week dietary study, chlorothalonil (purity unspecified) was fed at 0, 250, 500 or 750 mg/kg to groups of four beagle dogs. There were no compound-related effects on appearance, behaviour, appetite or body weight. No changes in haematological parameters were found at weeks 0, 4, 13 or 16. At termination, protein-bound iodine was found to be increased in all treated dogs. Urinalysis at weeks 6, 9, 13 and 16 was unremarkable. No compound-related macroscopic or microscopic changes were found at necropsy. In particular, only incidental changes were observed in liver and kidneys. A NOAEL was not established in this study (Paynter & Murphy, 1967; FAO/WHO, 1993b).

7.2.2 Dermal: Rabbit

Chlorothalonil (75% formulation) was applied to the intact or abraded skin of groups of rabbits at 0, 500 or 1000 mg/kg per day, 5 days/week, for 3 weeks. The treated groups comprised five males and five females per group, with two males and two females as controls. Treatment resulted in dose-related irritation which was more severe with abraded skin. Histopathological examination revealed a moderate degree of acanthosis, hyperkeratosis and slight to moderate leucocytic infiltration. No abnormality was detected in other tissues (Paynter, 1965b).

Chlorothalonil was applied dermally each day for 21 days to groups of six male and six female rabbits at 0.1, 2.5 and 50 mg/kg per day. The fungicide was suspended in 0.125% aqueous methylcellulose and covered 10% of the body surface when applied to the back. Contact was for 6 h daily. The only effect revealed by a wide range of observations and examinations was dermal irritation at 2.5 and 50 mg/kg per day. The histopathological changes seen were minimal to slight acanthosis and hyperkeratosis. The no-observed-effect level (NOEL) for dermal irritation was 0.1 mg/kg per day (Shults et al., 1986).

7.3 Long-term exposure

7.3.1 Rat

An early study, lasting 76 weeks, produced evidence of kidney changes in rats (15 male and 15 female per group) fed high levels of chlorothalonil at 500, 1000 and 5000 mg/kg diet. There was no overall effect on mortality, clinical condition or growth. The

compound-related changes in the kidney, at all levels, consisted of tubular hypertrophy, epithelial irregularities and vacuolation. Males were more affected than females (Paynter & Busey, 1967).

A 2-year study, reported in 1967, was initiated with chlorothalonil levels of 1500, 15 000 and 30 000 mg/kg diet with groups of 35 male and 35 female rats (70 of each sex in control group). Because of cathartic effects, the top dose administration was curtailed after 15 weeks. The rats in the two remaining treatment groups continued for the full 2 years with evaluations including haematology, biochemistry and histopathology. There was no effect on survival, but the effect on growth was dose-related. The relative organ weights of liver and kidney were increased at 15 000 mg/kg. Microscopic changes in the forestomach were described as acanthosis and hyperkeratosis of the squamous epithelium at 15 000 mg/kg and, in the kidney, as tubular hypertrophy and hyperplasia at both 1500 and 15 000 mg/kg diet (Paynter, 1967a).

A supplementary study, run concurrently with the previous study, assessed chronic toxicity at 5000 mg/kg diet. Growth rate was depressed and a cathartic action was expressed as increased water consumption and faecal excretion. Relative organ weights were increased for caecum and kidneys. Histopathological examination showed kidney changes as tubular hypertrophy and occasional degeneration of the proximal tubular epithelium (Paynter & Crews, 1967).

A 2-year study at six chlorothalonil dose levels between 4 and 60 mg/kg diet was designed to determine an NOEL (50 males and 50 females per group). No effects were seen on survival, clinical observations, growth, haematological or biochemical parameters. No changes of toxicological significance were found for organ weights or during gross and microscopic examination of tissues. The highest dose level (3 mg/kg body weight per day) was considered the NOEL (Holsing & Shott, 1970).

A long-term study was undertaken to evaluate the carcinogenic potential of chlorothalonil in Fischer-344 rats. Males were studied for 27 months and females for 30 months. Chlorothalonil was administered in the diet to groups of 60 males and 60 females at dose levels of 0, 40, 80 and 175 mg/kg body weight per day. There was no effect on survival of females or males up to 2 years. However, at the highest dose level, there was increased mortality after 2 years, but only in males. Decreases in body weight gain

were dose-related at 80 and 175 mg/kg per day. Treatment-related effects on other parameters appeared to be related to nephrotoxicity. These included increases in serum urea nitrogen and creatinine, increased urine volume and decreased specific gravity, and increased kidney weight. Histopathological examination of the kidney showed a dose-related increase in chronic glomerulonephritis (nephropathy) compared with controls. A re-examination of the kidneys also revealed tubular hyperplasia (a sign of preneoplastic change) and chronic progressive nephropathy in the treated groups. Secondary lesions in other organs included periarteritis and parathyroid hyperplasia. There was increased incidence or severity of hyperplasia and hyperkeratosis of the squamous mucosa of the oesophagus and forestomach in all dosed groups, which was probably the result of the irritant effect of chlorothalonil. Neoplastic changes in the kidney and forestomach are evaluated in section 7.7 (Wilson et al., 1985b, 1986a).

A further study was carried out to evaluate the neoplastic findings in the kidney and stomach seen in the previous study and to determine a NOEL for non-neoplastic effects. Groups of 65 male and 65 female rats were administered chlorothalonil in the diet at doses of 0, 1.8, 3.8, 15 and 175 mg/kg body weight per day. Males were killed at 23 months (highest dose) and 26 months and females at 29 months. The effects at 175 mg/kg per day were similar to those described above, i.e. changes associated with the kidney and stomach. At 15 mg/kg per day there was elevated serum urea nitrogen and slightly increased kidney weight. At 3.8 mg/kg per day there was a small increase in kidney weight, but there were no effects at 1.8 mg/kg per day. Microscopic examination revealed an increased incidence and severity of epithelial hyperplasia in the proximal convoluted tubules at 3.8 mg/kg per day or more. Clear cell hyperplasia of the proximal convoluted tubules was increased at 15 mg/kg per day in females and at 175 mg/kg per day in both sexes. In addition, at 3.8 mg/kg per day or more, there was an increased incidence and severity of hyperplasia, hyperkeratosis, ulcers and erosions of the squamous mucosa of the forestomach. At 175 mg/kg per day, the incidence of erosions of the glandular stomach was significantly increased compared to controls. Renal tumours at levels of 15 and 175 mg/kg per day and stomach tumours at 3.8 mg/kg per day or more are evaluated in section 7.7. An NOEL of 1.8 mg/kg body weight per day was established for non-neoplastic effects seen in the study (Wilson et al., 1989a).

7.3.2 Mouse

A 2-year mouse study was carried out with 60 males and 60 females per group at 0, 750, 1500 and 3000 mg chlorothalonil/kg in diet. There was a slightly increased mortality in males at the highest dose level but no effect was seen on body weight, food consumption, clinical condition or haematological parameters. Kidney weight was increased in all treated groups compared to controls. Non-neoplastic changes in the kidney were characterized as glomerulonephritis, cortical tubular degeneration and cysts. The incidence of these changes, found at all treatment levels, was not dose-related but was considered to be due to treatment. A histopathological re-evaluation of the kidneys revealed a high incidence of tubular hyperplasia in all male groups and a lower incidence in females. Non-neoplastic effects in the stomach and oesophagus included hyperplasia and hyperkeratosis of the squamous mucosa, probably due to the irritant action of chlorothalonil. The evaluation of kidney and stomach tumours found in this study is described in section 7.7 (Wilson et al., 1983b, 1986b).

A second mouse study was undertaken to determine the NOEL for stomach and kidney changes in male mice. Sixty males per group were fed chlorothalonil at levels of 0, 15, 40, 175 and 750 mg/kg diet for 2 years. Kidney weight and incidence of tubular hyperplasia were increased at 750 mg/kg and, very slightly, at 175 mg/kg. The increased incidence of hyperplasia and hyperkeratosis of the forestomach was dose-related between 40 and 750 mg/kg. The dietary NOEL for non-neoplastic effects was determined to be 15 mg/kg (1.6 mg/kg body weight per day). The tumour evaluation is considered in section 7.7 (Wilson et al., 1987).

7.3.3 Dog

In a 2-year study, chlorothalonil (93.6% purity) was fed to groups of four beagle dogs at dietary concentrations of 0, 1500, 15 000 or 30 000 mg/kg (equivalent to 0, 37.5, 375, or 750 mg/kg body weight per day). Eight dogs, one of each sex and of each group, were killed at 12 months and the remainder at 24 months. One dog of each treatment group lost weight during the study. There was a tendency towards mild anaemia in four of the mid-dose dogs at 2 years and at earlier intervals in two of the high-dose dogs. Biochemical and urine analyses were unremarkable. Absolute and relative thyroid and kidney weights, and liver to body weight ratios were increased at the mid- and high-dose

levels. Histopathological treatment-related changes occurred in the liver, thyroid, kidney and stomach of mid- and high-dose dogs; changes in low-dose dogs were equivocal. In the liver, the findings were similar in nature (though slightly more pronounced) at low-dose levels to those in the control dogs, but they increased in severity at mid- and high-dose levels. They included pericholangitis with associated portal fibrosis, bile duct hyperplasia and pigmentation of hepatic cytoplasm and of macrophages of sinusoids and portal triads. Renal glomerulosclerosis and degenerative renal tubular changes (tubular hypertrophy and dilation) were found in the kidneys of mid- and high-dose dogs. In the thyroid, markedly increased pigmentation of follicular epithelia occurred in mid- and high-dose dogs. Moderate to severe gastritis was found irregularly in mid- and high-dose animals. In summary, administration of chlorothalonil in the diet of dogs at concentrations of 15 000 and 30 000 mg/kg caused irregular body weight reduction, borderline anaemia and histopathological changes in the liver, kidney, thyroid and stomach. At 1500 mg/kg, the histopathological changes found in the liver were qualitatively similar but minimally to slightly increased in comparison to those found in control animals. Histopathological changes to the other tissues were unremarkable at the low dose. A NOAEL was not established in this study (Paynter & Busey, 1966; FAO/WHO, 1993b).

Groups of beagle dogs (eight males and eight females per group) were fed chlorothalonil in the diet at dose levels of 0, 60 and 120 mg/kg. Four dogs of each sex per group were killed at one year and the remaining animals at 2 years. There were no effects on behaviour or growth over the course of the study. Clinical chemistry values, including haematological, biochemical and urine analyses, were comparable to the controls at all dose levels. Gross and microscopic examination of tissues and organs performed on animals killed at 12 months indicated a slight increase in the severity of renal tubule vacuolation in high-dose males. Examination of tissues and organs at 24 months showed a slight degree of renal tubule vacuolation in two out of four males at 120 mg/kg. In the absence of other changes (urinalyses values) this finding was considered questionable, especially as a slight degree of vacuolation was noted in controls as well as other treated animals. The NOAEL was considered to be 120 mg/kg diet, equivalent to 3 mg/kg body weight per day (Holsing & Voelker, 1970; FAO/WHO, 1991, 1993b).

7.3.4 Summary of key dietary studies

A summary of the key dietary studies with chlorothalonil is given in Table 10.

7.4 Skin and eye irritation; sensitization

Chlorothalonil is an irritant to rabbit skin, as shown by repeated dose studies (5 days/week for 3 weeks at 500 or 1000 mg/kg per day or daily for 21 days at 2.5 or 50 mg/kg per day; details in section 7.2.2).

The influence of the vehicle on the skin irritant potential of chlorothalonil was shown by a rabbit study with 0.1% chlorothalonil in saline, petrolatum or acetone. Compared to the vehicle alone, chlorothalonil did not cause a significant increase in irritation in saline, but doubled the mild irritation caused by petrolatum. Acetone itself caused no irritation but the addition of chlorothalonil produced mild skin irritation (Flannigan & Tucker, 1985).

A further study in rabbits using a cumulative irritation assay confirmed the irritant properties of 0.1% chlorothalonil in acetone. A concentration of 0.01% gave evidence of mild irritation, probably of no clinical significance, and 0.001% was not irritant to the skin (Flannigan et al., 1986).

Chlorothalonil irritancy to the eye was evaluated in a modified Draize system, 0.1 mg being instilled into one eye of each of three male and three female rabbits. The eyes were examined and the results scored after 24, 48 and 72 h, 7 and 14 days. Ocular irritation occurred in all animals and corneal opacity persisted to day 14 (Major et al., 1982).

Skin sensitization was tested in a guinea-pig maximization test using 10 Hartley female guinea-pigs. Topical concentrations of 0.5 and 5% chlorothalonil were used. Upon challenge, chlorothalonil was shown to be a strong sensitizer. Moderate cross-sensitization with benomyl was also demonstrated (Matsushita & Aoyama, 1981). By contrast, chlorothalonil did not produce skin sensitization in a Draize test. The test substance (0.2 g) was applied to the shaved backs of Hartley-derived guinea-pigs. The material was occluded for 24 h and then removed. This procedure was performed 3 times a week for a total of 10 applications. On day 36 of the study a challenge application of 0.2 g chlorothalonil

Table 10. Summary of key dietary studies with chlorothalonil

Species	Duration	Dose levels	NOEL	LOEL	Key effects	Reference
Rat	13 weeks	0, 1.5, 3, 10, 40 mg/kg body weight per day	3 mg/kg per day	10 mg/kg per day	40 mg/kg per day: increased kidney and liver weight, incidence of hyperplasia and hyperkeratosis of forestomach, incidence of epithelial hyperplasia in proximal tubules 10 mg/kg per day: increased kidney weight, hyperplasia and hyperkeratosis of forestomach	Wilson et al. (1983a, 1985a)
Rat	23-29 months	0, 1.8, 3.8, 15, 175 mg/kg body weight per day	1.8 mg/kg per day	3.8 mg/kg per day	175 mg/kg per day: increases in serum urea nitrogen, urine volume, kidney weight, renal tubular hyperplasia, hyperplasia and hyperkeratosis of forestomach; increased incidence of renal and forestomach tumours 15 mg/kg per day: similar but less intense changes to those shown at highest dose level 3.8 mg/kg per day: small increase in kidney weight, renal tubular hyperplasia, hyperplasia and hyperkeratosis of forestomach and forestomach tumours	Wilson et al. (1989a)

Table 10 (contd).

Mouse	13 weeks	0, 7.5, 15, 50, 275, 750 mg/kg diet	15 mg/kg diet (= 3 mg/kg per day)	50 mg/kg diet (= 10 mg/kg body weight per day)	275 and 750 mg/kg: increased kidney weight 50, 275 and 750 mg/kg: dose-related increased incidence of hyperplasia and hyperkeratosis of forestomach	Shults et al. (1983); Bussey (1985)
Mouse	2 years	0, 15, 40, 175, 750 mg/kg diet	15 mg/kg diet (= 1.6 mg/kg body weight per day)	40 mg/kg diet (= 4.5 mg/kg body weight per day)	750 mg/kg: increased kidney weight, renal tubular hyperplasia, forestomach hyperplasia and hyperkeratosis, slightly increased incidence of forestomach tumours 175 mg/kg: slightly increased incidence of renal tubular hyperplasia, increased forestomach hyperplasia and hyperkeratosis 40 mg/kg: increased incidence of forestomach hyperplasia and hyperkeratosis	Wilson et al. (1987)
Dog	2 years	0, 1500, 15 000, 30 000 mg/kg diet	-	1500 mg/kg in diet (= 37.5 mg/kg body weight per day)	15 000 and 30 000 mg/kg: increased kidney, liver and thyroid weights and histopathological changes, gastritis 1500 mg/kg: slightly increased incidence of hepatic findings	Paynter & Bussey (1966)
Dog	2 years	0, 60, 120 mg/kg diet	120 mg/kg diet (= 3 mg/kg body weight per day)	-	no changes of toxicological significance	Holsing & Voelker (1970); FAO/WHO (1991, 1993)

was applied to the shaved flanks and occluded. The skin was assessed 24 h later after removal of the occlusive dressing. The positive control substance DNCB showed the expected dermal sensitization but chlorothalonil was shown not to be a sensitizer in this test (Wilson et al., 1982).

Luperi & Forster (1988) studied the ability of chlorothalonil to induce delayed contact hypersensitivity in the guinea-pig using the maximization test of Magnusson and Kligman. There was no evidence of an induced sensitization response, but adequate evaluation was impeded by a diffuse irritant reaction following the challenge.

7.5 Reproductive and developmental toxicity

Teratological evaluations have been carried out in the rat and rabbit.

Chlorothalonil was administered orally via gavage to pregnant Sprague-Dawley rats (25 per group) on days 6-15 of gestation at dose levels of 0, 25, 100 or 400 mg/kg body weight per day, and the animals were killed on day 20. Clinical signs of maternal toxicity were evident at the highest dose level. There were 3 deaths and lowered body weight during the dosing period. There was a slight increase in the number of early embryonic deaths at the highest dose level, probably associated with the maternal toxicity. There were no compound-related incidences of external, internal or skeletal malformations in the fetuses in treated animals. It was concluded that chlorothalonil is not teratogenic to the rat (Mizens et al., 1983).

Chlorothalonil was given orally to pregnant rabbits on days 8-16 of gestation at dose levels of 0, 180 or 375 mg/kg per day (days 8 and 9) and 0, 62.5 or 31.25 mg/kg per day (day 10 to day 16). There were marked effects on food intake and maternal mortality occurred in both treated groups, which imposed limitations on the evaluation of the study. However no teratogenic effect was observed (Paynter, 1966b).

Rabbits were dosed with chlorothalonil (0, 5, or 50 mg/kg per day) during days 6-18 of pregnancy (8 in control group, 9 in each dose group) and killed on day 29. Four out of nine does aborted at the highest dose level, and body weight was reduced in this group. Although the incidence of fetal deaths appeared to increase with dose, the difference was not statistically significant. The number

of implants and live fetuses per pregnancy and the fetal weights were reduced in the high-dose group compared to controls. No treatment-related effects were seen during external, internal or skeletal examinations. It was concluded that chlorothalonil was not teratogenic to the rabbit (Shirasu & Teramoto, 1975).

Chlorothalonil was administered by gavage to groups of 20 pregnant rabbits on days 7-19 of gestation at dose levels of 0, 5, 10 or 20 mg/kg per day. All survivors were weighed, killed on day 30 and examined for live, dead or resorbed fetuses. Live and dead fetuses were weighed and examined for external, visceral and skeletal abnormalities. The highest dose level caused maternal body weight loss and decreased food consumption. The pregnancy rate was $\geq 95\%$ in each group. Examinations revealed no fetotoxicity or teratogenicity due to chlorothalonil (Wilson et al., 1988).

A three-generation reproduction study was undertaken in rats at dietary levels of 0, 1500 or 15 000 mg chlorothalonil/kg diet. A supplementary study with 0 or 5000 mg/kg was also carried out 6 months later. Groups consisted of 10 males and 20 females which were fed the test diet for 11 or 12 weeks prior to mating. The study design was for three generations with two matings per generation to give A and B litters. Growth suppression occurred in the nursing A and B litters in all generations at all dose levels and the pups appeared smaller than the controls. There were no malformations due to treatment. However, there were difficulties in execution, and this study is not considered adequate by present-day standards (Paynter, 1967b).

Reproductive performance was also assessed in a one-generation study on rats at dietary levels of 0, 200, 375, 750, 1500 or 3000 mg/kg diet with groups of 15 males and 15 females. The parents were treated for 10 weeks prior to mating. No clinical signs of toxicity were evident in the parents, but male body weight gain was reduced at 1500 and 3000 mg/kg and the kidneys were enlarged at 3000 mg/kg. Reproductive parameters such as mating, fertility and gestation length were not affected. There were no treatment-related abnormalities in the offspring. The only effect on the offspring was lower body weight at 3000 mg/kg on lactation days 14 and 21 (Wilson et al., 1989b).

In a two-generation reproduction study with two litters per generation, technical chlorothalonil was administered to Charles River CD rats by dietary admixture at concentrations of 0, 500,

1500 and 3000 mg/kg diet. There were 35 rats of each sex in each group. The parental animals from each generation were treated continuously during the growth period prior to mating and then throughout the mating, gestation, lactation and resting phases of the study. The growth period was 10 weeks for the F₀ animals and 14 weeks for the F₁ animals. Each generation of parental animals was mated twice to produce the F_{1a}, F_{1b}, F_{2a} and F_{2b} litters. The offspring were exposed to the test diets throughout the lactation period. Thirty-five F_{1b} males and females per group were selected to become the F₁ generation after weaning. No mortalities or clinical signs of toxicity associated with administration of chlorothalonil were observed in the F₀ or F₁ parent animals. There was a treatment-related effect towards lowered body weight in both males and females in the F₀ and F₁ adults. The NOEL for body weight was 500 mg/kg in diet. Increased relative food consumption was observed in the groups that showed lower body weights. The anticipated treatment-related lesions in the kidneys and stomachs of adult animals were observed in the F₀ and F₁ generations by gross and microscopic pathology. These effects were observed in the kidney at all dose levels in males and at 1500 and 3000 mg/kg in females. Stomach effects were observed at all dose levels in both sexes. Reproductive parameters in F₀ and F₁ animals, including mating and fertility indices and gestation length, were not affected by treatment with chlorothalonil. No gross malformations which were considered treatment-related were observed in offspring in any of the groups throughout the study. Litters were culled at day 4 to 8 pups/litter. No effects on the number of live and stillborn pups, pup sex ratio, pup survival and physical condition of the pups during lactation were observed. No findings which were considered treatment-related were observed during necropsy of the pups. The only effect observed in pups in this study was lowered body weight compared to the controls on day 21 of lactation. The NOEL for this effect was considered to be 1500 mg/kg diet, equal to 75 mg/kg body weight per day (Lucas & Benz, 1990).

7.6 Mutagenicity

Chlorothalonil has been assessed for mutagenic potential in a wide range of *in vitro* and *in vivo* assays as shown in Tables 11 and 12. Most of the tests showed chlorothalonil not to be mutagenic or clastogenic. A positive result in the DNA repair test with *Salmonella typhimurium* was not reproduced in *Bacillus subtilis*, nor was DNA binding shown in an *in vivo* study (see section 6.5). A positive result in Chinese hamster ovary cells without metabolic

activation was not seen in the presence of activation nor confirmed by *in vivo* chromosomal tests in rats and mice. Equivocal results were obtained with Chinese hamster bone marrow *in vivo*.

In addition to the results shown in the Tables 11 and 12, a series of studies was reported by IARC (1983). The results, which were all negative, included those with *S. typhimurium* in the presence and absence of a metabolic activating system and *Saccharomyces cerevisiae* and *Aspergillus nidulans* in the presence of activation. Chlorothalonil also failed to induce mutations in silkworms and chromosomal aberrations in barley shoot tips or hamster lung fibroblasts.

Chlorothalonil is not a transforming agent in Fischer rat embryo cell lines (Price, 1978a).

The monothio, dithio, trithio, dicysteine, tricysteine and monogluthathione metabolites of chlorothalonil have been shown to be negative in the Ames assay with or without rat kidney metabolic activation (see section 7.9).

Considering all the results of mutagenicity testing, it is unlikely that chlorothalonil will show mutagenic activity in intact mammalian systems.

7.7 Carcinogenicity

Several long-term rodent studies have been carried out on chlorothalonil and have included carcinogenic evaluation. The design of these studies and the chronic toxicity results have been described in section 7.3. Some of the earlier rat studies did not show any carcinogenic effect but it is probable that their design was not sufficient to provide a critical test. The carcinogenic evaluation in this section therefore concentrates upon the more recent rat and mouse studies. Detailed evaluations of these studies have already appeared in the Joint FAO/WHO Meeting on Pesticide Residues (JMPR) reviews of 1983, 1985 and 1990 (FAO/WHO, 1985, 1986b, 1990b). Therefore, for most of these studies, only the salient points will be described here.

A bioassay of technical grade chlorothalonil for possible carcinogenicity was conducted by administering the test chemical in the diet to Osborne-Mendel rats and B6C3F₁ mice. Groups of 50 rats of each sex were administered chlorothalonil at one of two dose levels for 80 weeks and then observed for 30-31 weeks.

Table 11. *In vitro* mutagenicity tests

Test	Organism	Metabolic activation (+ or -)	Dose range	Mutagenic potential	Reference
Prokaryotes Point mutation	<i>Salmonella typhimurium</i> (5 strains)	+ and -	0.33-6.6 µg/plate	negative	Benzer (1977a)
Point mutation	<i>S. typhimurium</i> (5 strains)	+ and - (renal)	0.16-50 µg/plate	negative	Jones et al. (1984)
Point mutation	<i>S. typhimurium</i> (5 strains)	- +	1-10 µg/plate 2-10 µg/plate	negative negative	Shirasu et al. (1977)
	<i>Escherichia coli</i> WP2	- +	10-500 µg/plate 10-100 µg/plate	negative negative	
Point mutation	<i>S. typhimurium</i> TA98, TA100, TA1535, TA1537, TA1538	+ and - (hepatic and renal)	0.76, 7.6, 76 µg/plate	negative	Wei (1982)

Table 11 (contd).

DNA repair tests	<i>S. typhimurium</i> TA1978, TA1538	+ and -	2, 10, 20 µg	positive	Banzer (1977b)
	<i>Bacillus subtilis</i> H17/M45 rec-assay	-	2-200 µg	negative	Shirasu et al. (1977)
Mammalian cells Gene mutation	Chinese hamster V79	+ and -	0.3 µg/ml	negative	Banzer (1977c)
	mouse fibroblast BALB/3T3	+ and -	0.03 µg/ml	negative	
Chromosome aberration	Chinese hamster ovary cells	- +	0.03-0.3 µg/ml 0.6-6.0 µg/ml	positive negative	Mizens et al. (1986a)
Chromosome aberrations	Human lymphocytes	- +	0.54-2.5 µg/ml 1.16-5.38 µg/ml	negative negative	Mosesso & Forster (1988)

Table 12. *In vivo* mutagenicity tests

Species	Test	Dose	Mutagenic potential	Reference
Mouse	<i>in vivo</i> cytogenetics	6.5 mg/kg per day 5 days orally	negative	Legator (1974a)
Mouse	host-mediated assay; 8 strains <i>Salmonella typhimurium</i>	6.5 mg/kg per day 5 days orally	negative	Legator (1974a)
Mouse	dominant lethal assay, 5 days dosing, 8 weeks mating	6.5 mg/kg per day 5 days orally	negative	Legator (1974a)
Mouse	micronucleus (polychromatic erythrocytes)	4-2500 mg/kg orally, twice with 24 h interval	negative	Siou (1981a)
Mouse	chromosome aberration (bone marrow 6 h after last dose) (bone marrow at 6, 24 and 48 h)	4-2500 mg/kg orally, twice with 24 h interval 250, 1250, 2500 mg/kg oral single dose	negative negative	Siou (1981b) Mizens et al. (1985a)

Table 12 (contd).

Rat	micronucleus (polychromatic erythrocytes)	8-5000 mg/kg orally, twice with 24 h interval	negative	Siou (1981a)
Rat	chromosome aberration (bone marrow 6 h after last dose) (bone marrow 6, 24 and 48 h)	doses as above 500, 2500, 5000 mg/kg oral single dose	negative negative	Siou (1981b) Mizens et al. (1985b)
Chinese hamster	micronucleus (polychromatic erythrocytes)	4-2500 mg/kg orally, twice with 24 h interval	negative	Siou (1981a)
Chinese hamster	chromosome aberration (bone marrow 6 h after last dose) (bone marrow at 6, 24 and 48 h) (bone marrow 6 h after last dose)	8-5000 mg/kg for 2 days 500, 2500, 5000 mg/kg oral single dose 50, 125, 250 mg/kg per day orally for 5 days	inconclusive equivocal (± at 48 h) weak response not dose-related	Siou (1981b) Mizens et al. (1985c) Mizens et al. (1985c)

Time-weighted average doses for both males and females were 5063 or 10 126 mg/kg diet. Matched controls consisted of groups of 10 untreated rats of each sex; pooled controls consisted of the matched control groups combined with 55 untreated male or female rats from similar bioassays of five other test chemicals. All surviving rats were killed at 110-111 weeks. Groups of 50 mice of each sex were administered chlorothalonil at one of two dose levels for 80 weeks, then observed for 11-12 weeks. Time-weighted average doses for males were 2688 or 5375 mg/kg diet, and for females, 3000 or 6000 mg/kg diet. Matched controls consisted of groups of 10 untreated mice of each sex; pooled controls consisted of the matched control groups combined with 50 untreated male or female mice from similar bioassays of five other test chemicals. All surviving mice were killed at 91-92 weeks. Clinical signs that appeared with increasing frequency in dosed rats included haematuria and, from week 72 until termination of the study, bright yellow urine. Since the dosed female mice did not have depression in mean body weights or decreased survival compared with the controls, they may have been able to tolerate a higher dose. In rats, adenomas and carcinomas of the renal tubular epithelium occurred with a significant dose-related trend in both the males and females (males: pooled controls 0/62, low dose 3/46, high dose 4/49; females: pooled controls 0/62, low dose 1/48, high dose 5/50). These tumours included both adenomas and carcinomas which are considered to be histogenically related. In mice, no tumours were found to occur at a greater incidence among dosed animals than among controls. It was concluded that under the conditions of this bioassay, technical grade chlorothalonil was carcinogenic to Osborne-Mendel rats, producing tumours of the kidney. However, chlorothalonil was not carcinogenic for B6C3F₁ mice (US NCI, 1978).

In a lifetime study on Fischer-344 rats at dietary doses of 0, 40, 80 or 175 mg/kg body weight per day (see section 7.3), a higher incidence of primary renal tumours of epithelial origin (adenomas and carcinomas) was seen in the treated groups (0/60, 7/60, 7/60 and 19/60 for males and 0/60, 3/60, 6/60 and 23/60 for females). It was considered that the increased incidence of renal hyperplasia seen in this study was associated with the formation of these tumours and constituted a pre-neoplastic change. Papillomas and carcinomas of the squamous mucosa of the forestomach were found in rats in the treated groups (0/60, 1/60, 1/60, 3/60 for males and 0/60, 1/60, 2/60, 7/60 for females). These are probably related to the proliferative non-neoplastic effects on the squamous

mucosa as a result of the chronic irritation by chlorothalonil (Wilson et al., 1985a, 1986b).

A further dietary study evaluated the carcinogenicity of chlorothalonil at lower doses of 1.8, 3.8 and 15 mg/kg body weight per day as well as at 175 mg/kg body weight per day (see section 7.3). Rats with renal tumours (adenomas and carcinomas) occurred at 1/55, 1/54, 1/54, 4/54, 23/55 (male groups) and 0/55, 0/54, 0/55, 0/53, 32/55 (female groups). This confirmed the effect at 175 mg/kg per day and an NOEL of 3.8 mg/kg per day was determined for these tumours. Animals with papillomas and carcinomas of the forestomach occurred at 0/55, 0/54, 3/54, 2/54, 5/55 (male groups) and 1/55, 1/54, 2/55, 5/53, 9/55 (female groups) giving an NOEL of 1.8 mg/kg per day (Wilson et al., 1989a).

A 2-year study with Charles River CD-1 mice at 750, 1500 or 3000 mg chlorothalonil/kg diet (section 7.3) showed increased incidences of gastric and renal tumours in the treated groups. Mice with tumours of the squamous epithelium of the forestomach occurred at 0/60, 2/60, 5/60, 2/60 (male groups) and 0/60, 2/60, 4/60, 5/59 (female groups). Although not strictly dose-related, these results were considered to be a treatment effect and linked to the irritant properties of chlorothalonil. There was also a low incidence of renal tubular tumours in male treated groups, not seen in controls, at 0/60, 6/60, 4/60, 5/60 (not dose-related). These were probably linked to the high incidence of renal tubular hyperplasia seen in male mice in the treated groups (Wilson et al., 1983b, 1986b).

A second study at 0, 15, 40, 175 and 750 mg/kg diet was undertaken to establish an NOEL for kidney and stomach changes in male mice (section 7.3). Only two renal tumours (one at 40 mg/kg and one at 175 mg/kg) were found. There was a slightly higher incidence of squamous tumours of the forestomach at 750 mg/kg. Taking account of the overall results of the two studies it was considered that the tumorigenic NOEL was at least 175 mg/kg diet. In this study, the NOEL for tubular hyperplasia was 40 mg/kg (equal to 4.5 mg/kg per day) and the NOEL for hyperplasia/hyperkeratosis in the forestomach was 1.6 mg/kg per day (Wilson et al., 1987).

7.8 Other special studies

Rats fed 0, 1500 or 15 000 mg chlorothalonil/kg diet showed a dose-related decrease in the retention of a dye, indicating a

laxative effect. A more detailed study attempted to determine the effect of chlorothalonil on the absorption and utilization of proteins, fats and amino acids during a 10-week feeding study on a group of 10 male and 10 female rats. It was concluded that the compound did not interfere directly with the absorption and utilization and that the depressed weight gain was probably due to catharsis (Paynter, 1967c).

In a study by Andre et al. (1991), mitochondria were obtained from fresh kidney cortical tissue by homogenization and differential centrifugation. The mitochondria were incubated in the presence or absence of sulfur-containing analogues of chlorothalonil and the degree of mitochondrial respiratory control was evaluated by polarographic techniques. The following sulfur-containing analogues of chlorothalonil were tested: the mono-, di-, and tri-thiol analogues and the mono-, di-, and tri-glutathione analogues. Kidney mitochondria were incubated with succinate, a site 2 substrate, or with glutamate, a site 1 substrate, in the presence or absence of the test material. Mitochondrial respiratory control, expressed as the acceptor control ratio (ACR), was determined by taking the ratio of the rate of oxygen consumption in the presence of ADP (state 3) to the rate of oxygen consumption after the ADP had been consumed (state 4). When the mono-thiol, mono-, di-, or tri-glutathione analogues of chlorothalonil and succinate were added to kidney mitochondria, no significant differences were found in the ACR from the controls. Incubation of the di- or tri-thiol analogues of chlorothalonil and succinate with kidney mitochondria resulted in significant differences of the experimental ACR from the control ACR. When glutamate was used as the substrate for the electron transport system in kidney mitochondria, no significant differences from the control were detected for any of the six test materials. These data suggest that the effects of the di- or tri-thiol analogues of chlorothalonil may impair the respiratory control of kidney mitochondria by inhibiting the transfer of reducing equivalents from succinate to coenzyme Q. The effects on mitochondrial respiration may be due to the formation of disulfide bonds between the thiol analogues and proteins.

7.9 Toxicity of metabolites

Most studies have centred on the 4-hydroxy-2,5,6-trichloro-isophthalonitrile metabolite. This is found as a small proportion of chlorothalonil plant residues (section 4.2.1) and is also a breakdown product of chlorothalonil in the environment. It has

been identified in faeces of laboratory animals after chlorothalonil dosing. It is more acutely toxic than chlorothalonil itself (acute oral LD₅₀ values are 332 and 10 000 mg/kg, respectively).

Several laboratory animal studies have been undertaken with the 4-hydroxy metabolite and have been described in some detail in JMPR reviews (FAO/WHO, 1978, 1982, 1985). The following is a brief summary of the studies and their results.

Various effects were noted in rats fed the 4-hydroxy metabolite at eight dose levels (10 to 750 mg/kg body weight per day) for 61-69 days. Mortality was increased in males at 125 mg/kg per day or more, and in females at 75 mg/kg per day or more. Body weight was depressed in both sexes at ≥ 40 mg/kg per day. Anaemia was evident at 75 mg/kg per day or more in males and 40 mg/kg per day or more in females. Histopathological examination revealed treatment-related effects in bone marrow and spleen (in the form of erythroid hyperplasia and depressed granulopoiesis) at ≥ 40 mg/kg per day and in the liver (haemosiderosis, centrilobular hepatitis) and kidney (cortical atrophy) at ≥ 75 mg/kg per day. The overall NOEL was 20 mg/kg body weight per day (Murchison, 1979).

A rabbit teratology study was undertaken with oral doses of 0, 1, 2.5 and 5 mg/kg per day during days 6-18 of gestation, with necropsy at day 28. There was a marginal effect on dams at day 5 but no evidence of a teratogenic effect in the study (Wazeter & Goldenthal, 1976).

The 4-hydroxy metabolite was evaluated in a three-generation, two litters/generation study in groups of 15 male and 30 female rats at dose levels of 0, 10, 60 and 125 mg/kg diet. There were no treatment-related changes except for body weight reductions at 60 and 125 mg/kg (FAO/WHO, 1982).

In a one-generation follow-up study, groups of rats were fed diets containing the 4-hydroxy metabolite at 0, 10, 20, 30, 60 and 120 mg/kg diet for 18 weeks before mating (12 males and 24 females per group). Two sets of mating were undertaken. There was some effect on live pup weights at 60 and 120 mg/kg. The clear NOEL was considered to be 30 mg/kg diet (Ford, 1982).

The metabolite was assessed for chronic toxicity and carcinogenicity in long-term rodent studies. A 2-year rat study was undertaken at dose levels of 0, 0.5, 3 and 10 mg/kg body

weight per day with groups of 75 males and 75 females. Anaemia was evident at the highest dose. The NOEL was determined to be 3 mg/kg body weight per day. There was no evidence for a carcinogenic effect. In the mouse study, the dietary dose levels were 0, 375, 750 and 1500 mg/kg diet using groups of 60 males and 60 females. The study was terminated at 20-22 months because of increasing and high mortality. A series of effects, including amyloidosis, haemosiderin in the spleen and increases in reticulocyte counts and red cell haemolysis, precluded the establishment of an NOEL. No carcinogenic effect was evident (Hozan & Auletta, 1981; McGee, 1983).

The 4-hydroxy metabolite was not mutagenic in a number of *in vitro* and *in vivo* assays. These were the *Salmonella* mutagenicity assay with and without metabolic activation (Banzer, 1977d), a host-mediated assay in mice given a single intraperitoneal dose of 6.5 mg/kg (Legator, 1974b), Chinese hamster (V-79) and mouse fibroblast (Balb/3T3) cells in culture with and without activation (Banzer, 1977e), a micronucleus test in mice at 6.5 mg/kg per day for 5 days (Legator, 1974b), a dominant lethal study in male rats given single oral doses (0, 2, 4 or 8 mg/kg) singly or daily for 5 days (Hastings & Clifford, 1975), a dominant lethal study in male mice given 1, 3 or 6.5 mg/kg per day for 5 days (Legator, 1974b), a DNA repair assay using *S. typhimurium* in a spot test with or without activation (Banzer, 1977f), and a cell transformation assay with rat embryo cell lines in culture at 0.1, 1 or 10 µg/ml (Price 1978b).

A series of *in vitro* gene mutation assays with *S. typhimurium* tester strains with and without renal metabolic activation were undertaken with chlorothalonil, four manufacturing impurities and eight known or potential metabolites. No mutagenic potential was shown by any of the compounds. Full details were given in the 1985 JMPR evaluation (FAO/WHO, 1986b).

The mutagenic potential of the thiol and cysteine derivatives of chlorothalonil has been evaluated in the Ames test with and without metabolic activation with S9 from the kidney of male Fischer rats. These compounds were 2,5-dichloro-4,6-bismercaptoiso-phthalonitrile, 5-(2,4-dicyano-3,5,6-trichlorophenyl) glutathione, 5-chloro-2,4,6-trimercaptoisophthalonitrile, S,S'-(2,4-dicyano-3,6-dichlorophenyl)dicysteine and S,S',S''-(2,4-dicyano-6-chlorophenyl)-tricysteine (purity ranging from 90.5 to 97.5%). Four other compounds were used as positive controls. The *Salmonella typhimurium* tester strains TA98, TA100, TA1535,

TA1537 and TA1538 were used. In all these studies, there was no significant increase (doubling) over solvent control values in the number of revertants for any of the five tester strains used either with or without metabolic activation (Mizens et al., 1985d,e, 1986b,c, 1987).

A description of a 90-day rat study on the monogluthathione conjugate of chlorothalonil is given in section 7.3.

8. EFFECTS ON HUMANS

8.1 General population exposure

A case of acute facial dermatitis in a 53-year-old man, caused by staying in a summer cottage, has been reported. Patch testing revealed contact allergy to the paint that was applied to all the window-frames, and to the chlorothalonil contained in the paint. After removal of the frames, there were no further recurrences of facial dermatitis. The authors suggested that products containing chlorothalonil are not suitable for indoor use (Liden, 1990; Eilrich & Chelsky, 1991).

8.2 Occupational exposure

Chlorothalonil contact dermatitis was observed in a number of employees in a chlorothalonil manufacturing plant. There were 19 cases out of 103 employees. About 60% of the employees showed some kind of skin abnormality compared with 18.5% of employees not working with chlorothalonil. When the hygiene conditions of the plant were improved the overall proportion of skin abnormalities fell to about 20% and there were no cases of chlorothalonil contact dermatitis (Diamond Shamrock, 1980).

Wood preservatives containing chlorothalonil have also been implicated in the appearance of allergic contact dermatitis. One report concerned a Danish cabinet maker who developed dermatitis on his hands after 9 months of painting furniture with preservative containing the compound. This was possibly caused by contact via the wood dust after sandpapering (Bach & Pedersen, 1980). Another report referred to three cases, two with erythema on the face, particularly periorbitally, and one with eczema of the hands, in people engaged in similar work (Spindeldreier & Deichmann, 1980). The four people in these cases showed a positive reaction to patch tests with 0.01% chlorothalonil in acetone.

A further case of contact dermatitis was described by Meding (1986) in a 33-year-old male painter, who regularly worked with paint containing chlorothalonil. A patch test with chlorothalonil was positive.

Work-related skin complaints occurred in a Norwegian factory producing wooden window frames. The wood preservative used

was white spirit containing 0.5% chlorothalonil. Fourteen out of 20 workers experienced some kind of skin reaction including pruritus, erythema and oedema of the eyelids and other facial regions, and eruptions on arms and hands. Seven of these 14 subjects yielded a positive patch test reaction with 0.01% chlorothalonil in acetone compared with 1 out of 14 controls (Johnsson et al., 1983).

Allergic contact dermatitis has also been described in Japanese farmers (Horiuchi & Ando, 1980) and in Dutch horticultural workers (Bruynzeel & van Ketel, 1986) using chlorothalonil fungicide formulations.

In a group of 84 tea growers, two showed a positive skin patch test with 0.02% chlorothalonil in petrolatum (Fujita, 1985).

9. EFFECTS ON OTHER ORGANISMS IN THE LABORATORY AND FIELD

9.1 Laboratory experiments

9.1.1 Microorganisms

9.1.1.1 Aquatic microorganisms

The algicidal activity of chlorothalonil was examined by Goulding (1971). It was shown that this compound is effective against a range of algae including *Chlorella*, *Chlamydomonas*, *Ulothrix*, *Anabaena*, *Oscillatoria* and *Microcystis* at low concentrations, often less than 1 µg/litre. It was also effective on natural populations of algae obtained from lakes, rivers, and reservoirs. The effect was generally less after 300 h than after 150 h and was dependent upon the size of the initial cell inoculum.

Walker et al. (1984) describe a simple shake-flask screening test to evaluate pesticide persistence and aquatic toxicity in the laboratory. Four systems were used: active sediment, sterile sediment, active water and sterile water. Chlorothalonil at 162 µg/litre did not increase the mortality of *Mysidopsis* above the control level within 96 h. The authors concluded that degradation of chlorothalonil involved microorganisms and that the degradation products did not enhance the aquatic toxicity of chlorothalonil.

9.1.1.2 Soil microorganisms

Chlorothalonil, at dose levels up to 5000 mg/litre in bacterial suspensions, inhibited the growth of three strains of *Rhizobium japonicum* (Tu, 1980).

Chlorothalonil, at concentrations up to 1000 mg/kg medium, did not inhibit or stimulate the growth of any one of 25 strains of *Rhizobium* bacteria isolated from red clover root nodules (Heinonen-Tanski et al., 1982).

Several studies were conducted with chlorothalonil in soil to determine the effects (if any) on normal soil processes such as nitrogen fixation, nitrification and degradation of substrates such as protein, pectin, cellulose and starch. These studies were conducted at two rates: use rate (2.5 mg/kg) and 10 times the use

rate (25 mg/kg). In general, any inhibitory effects observed were temporary in nature and more pronounced at the high rate. No effects on the use of protein, pectin or cellulose by soil microorganisms were observed, but there was increased utilization of starch (Szalkowski et al., 1980).

The results obtained by Szalkowski et al. (1981a), who studied the effect of chlorothalonil on non-symbiotic nitrogen-fixing soil microorganisms, are given in Table 13.

Table 13. The effect of chlorothalonil on non-symbiotic nitrogen-fixing soil microorganisms

	Sandy loam		Clay loam	
	Application rate	Ten times application rate	Application rate	Ten times application rate
Aerobic nitrogen fixation	initial inhibition	inhibition at days 0, 21 and 28	no effect	stimulation
Anaerobic nitrogen fixation	general stimulation	inhibition up to day 21	no effect	stimulation

Szalkowski et al. (1981b) studied the effect of chlorothalonil on nitrogen transformation in sandy loam and clay loam soils at two rates: one equivalent to the application rate and one equivalent to ten times this rate. In sandy loam soil, there was a consistent inhibitory effect at the higher rate, which was reduced with time. In clay loam soil, at the higher rate, inhibition was no longer observed after day 21. In both types of soil at the normal rate, there was little if any inhibition.

9.1.2 *Aquatic organisms*

The acute toxicity of chlorothalonil to various aquatic species is shown in Table 14. Some of these toxicity tests were carried out

Table 14. Acute toxicity of chlorothalonil to aquatic organisms

Stage (weight or length)	Test systems	Freshwater/ marine (hardness)	pH	O ₂	Temperature (°C)	Solvent	Purity	48-h LC ₅₀ (µg/litre)	96-h LC ₅₀ (µg/litre)	Reference
Rainbow trout (<i>Oncorhynchus mykiss</i>)										
6-11 g	flow through	freshwater		80%	14	acetone	> 99%	19.0	17.1	Davies & White (1985)
6-11 g	flow through	freshwater		53%	16	acetone	> 99%	18.8	10.5	
6-11 g	semi-static (24 h)	freshwater		90%	10	acetone	> 99%		18.0	
-	static	freshwater (soft)		-	12	acetone	96%	56	49	SDS Biotech Corporation (1980a)
3.5-4.0 g	static	freshwater (12.3 mg/litre)	6.5-7.4	8.4-11.2 mg/litre	12.5-15.5	acetone	97.8%		76	Ernst et al. (1991)
3.5-4.0 g	static	freshwater (12.3 mg/litre)	6.5-7.4	8.4-11.2 mg/litre	12.5-15.5		Bravo 500		69	Ernst et al. (1991)
Bluegill (<i>Lepomis macrochirus</i>)										
-	static	freshwater (soft)			22	acetone	96%	46-77	62	SDS Biotech Corporation (1979)

Table 14 (contd).

Common jolly tail (<i>Galaxias maculatus</i>)									
7-10 g	flow through	freshwater	75%	16	acetone	99%	18.2	16.3	Davies & White (1985)
Spotted galaxias (<i>G. fruttaceus</i>)									
8-20 g	flow through	freshwater	75%	16	acetone	99%	25.8	18.9	Davies & White (1985)
Golden galaxias (<i>G. auratus</i>)									
7-11 g	flow through	freshwater	75%	13	acetone	99%	46.6	29.2	Davies & White (1985)
Three spine stickleback (<i>Gasterosteus aculeatus</i>)									
0.3 g	static	freshwater	7.7-8.0	9.2-9.5		Bravo 500		< 73	Ernst et al. (1991)
			mg/litre	9-10					

Table 14 (contd).

Stage (weight or length)	Test systems	Freshwater/ marine (hardness)	pH	O ₂	Temperature (°C)	Solvent	Purity	48-h LC ₅₀ (µg/litre)	96-h LC ₅₀ (µg/litre)	Reference
Channel catfish <i>Ictalurus punctatus</i>										
40-80 g	semi-static, 24 h	freshwater (30 mg/litre)	7.0-7.2	5.0-6.0 mg/litre	23	acetone	99%	62	52	Gallagher et al. (1992)
-	static	freshwater (soft)			22	acetone	96%	55	44	SDS Biotech Corporation (1980b)
Spot <i>Leiostomus xanthurus</i>										
-	flow through	brackish water (22 ppt)			11		technical	32		Mayer (1987)
Sheepshead minnow <i>Cyprinodon variegatus</i>										
3-7 days	static	marine					technical		32	SDS Biotech Corporation (1982b)

in static or semi-static systems and others in flow-through systems. In view of the strong adsorption and degradation characteristics of chlorothalonil, nominal concentrations in static systems are likely to underestimate the toxicity (overestimate the LC₅₀) of chlorothalonil. At the same time, it should be pointed out that the static test systems more closely resemble the field situation.

Davies & White (1985) described the reactions of fish to exposure. *Oncorhynchus mykiss* and *Galaxias* sp showed marked lethargy, the degree increasing with time and concentration of exposure. *O. mykiss* showed normal startle reactions at concentrations below 8.7 µg/litre, and *G. maculatus*, *G. truttaceus* and *G. auratus* at 8.8, 9.0 and 13.3 µg/litre, respectively, over 96 h. In *O. mykiss*, loss of startle reaction was followed by reduction of activity, and permanent lethargy was followed by loss of righting ability and death. In *Galaxias* sp, the onset of lethargy was accompanied by varying degrees of fin collapse.

Davies & White (1985) commented on the fact that most acute toxicity values have been obtained in tests where solvent was added to the chlorothalonil.

A 48-h EC₅₀ has been reported for the pink shrimp (*Penaeus duorarum*) at 320 µg/litre and a 96-h EC₅₀ for the eastern oyster (*Crassostrea virginica*) at 26 µg/litre. The value for the pink shrimp was based on immobility or loss of equilibrium and that for the eastern oyster on shell deposition (Mayer, 1987). Oysters suffered a 42% mortality when exposed to 1000 µg chlorothalonil per litre for 96 h and a 25% reduction in growth at 10 µg/litre (SDS Biotech Corporation, 1983b).

Ernst et al. (1991) used both laboratory bioassay and field treatments of a pond system to determine the toxic effects of chlorothalonil on aquatic fauna. The acute toxicity of technical chlorothalonil and a commercial formulation on five species, including mussel and clams, was determined (Table 14). In the field study also reported by O'Neill (1991) (see section 5.1.2 for measured concentrations after spraying), the mortality of caged invertebrate and vertebrate species was monitored. Seven species were caged in the stream flowing from the treated pond: water boatman (*Sigara alternata*), caddisfly larva (*Limnephilus* sp), freshwater clam (*Pisidium*, sp), crawling water beetle (*Halipplus* sp), scud (*Gammarus* spp.), stickleback (*Gasterosteus aculeatus*) and midge larva (*Chironomidae*). The floating cages were placed

near the surface of the water. The water boatman suffered the highest mortality, ranging from 49 to 84% in replicate cages; mortality in a control pond was 16-20% over the same period (24 h). Midge larva mortality (69%) was judged by the authors to be a consequence of handling. Stickleback mortality was 37 to 56% in the treated pond, compared to 2 to 6% in the controls. Caddisfly larvae, clams, beetles and scud showed no deaths during the 24 h. Rainbow trout (*Oncorhynchus mykiss*) also showed no mortality following spraying. An estimate of total invertebrate numbers before and after spraying in the pond showed a slight increase following the first spray and a slight reduction after the second (not statistically significant). The control pond also showed fluctuations. The total was heavily influenced by *Chironomid* midge larvae, which was by far the most frequently occurring species. This study showed that a lower toxicological effect was observed in the field study than in the laboratory bioassays, indicating a reduction in exposure to the available chlorothalonil and thus less severe impacts in the pond system through physical and chemical processes.

Gallagher et al. (1992) showed that sublethal chlorothalonil exposure may cause acute necrosis of the intestinal epithelial lining in channel catfish. Exposure to 13 µg/litre for 72 h resulted in increased tissue GSH concentrations in liver, posterior kidney and gills, which suggests a protective role for tissue GSH against chlorothalonil exposure.

When two generations of *Daphnia magna* were exposed to technical chlorothalonil at levels of 6.2, 12, 25, 50 and 100 µg/litre for 21 consecutive days during each generation, adverse effects on adult survival and reproduction were observed at a nominal concentration of 100 µg/litre. The maximum acceptable toxicant concentration (MATC) for technical chlorothalonil, based upon nominal concentrations, was 50 µg/litre (35 µg/litre was the measured concentration) (Shults et al., 1982).

Fathead minnows (*Pimephales promelas*) were continuously exposed in duplicate aquaria to nominal concentrations of 25, 12.5, 6.3, 3.1 and 1.5 µg/litre (measured concentrations 16, 6.5, 3.0, 1.4 and 0.6 µg/litre, respectively) technical chlorothalonil, a diluent water control, and a solvent (acetone) control throughout a complete (egg to egg) life cycle. No significant effects were observed in either generation at mean measured concentrations ≤ 3.0 µg/litre. The first generation (F₀) eggs exhibited a significantly reduced hatchability and survival of fry after 35 days

when exposed to a mean measured concentration of 16 µg/litre. The reproductive success of F₀ fish was adversely affected (reduction in the number of eggs per spawn) by exposure to concentrations ≥ 6.5 µg/litre. The second generation (F₁) eggs exhibited a significantly reduced hatchability when exposed to a mean measured concentration of 6.5 µg/litre. The survival of fry at this concentration was not affected. Based on these data, the MATC (mean measured) of technical chlorothalonil in water for fathead minnows was estimated to be in the range of 3.0 to 6.5 µg/litre (Shults et al., 1980).

9.1.3 Terrestrial organisms

9.1.3.1 Plants

In a study by Stephenson et al. (1980), 30-day-old tomato plants were treated with chlorothalonil (at 2.5 kg/ha) or chlorothalonil in combination with metribuzine. The effect was assessed as the tomato shoot dry weight (as a percentage of control plant weight). On this basis, the weight of the chlorothalonil-treated plants was 89% of the control plant weight, a difference which was statistically significant ($P > 0.05$). The effect was additive when chlorothalonil was used in combination with metribuzine.

9.1.3.2 Earthworms

When chlorothalonil suspension concentrate (500 g/litre) was added to artificial soil containing earthworms (*Eisenia foetida*), the treated soil was non-toxic after 7 and 14 days. The LC₅₀ was found to be > 1000 mg/kg soil (on a dry weight basis) (Wüthrich, 1990).

When earthworms (*Eisenia foetida*) were immersed for 1 min in solutions of chlorothalonil (0.1, 1 and 2% w/v); there was no effect on survival. Bermuda grass clippings were air dried and ground; 15 g samples were then stirred into 100 ml of 0.1% chlorothalonil and subsequently fed to earthworms after filtration of excess liquid. There was no effect on longevity. Worms reared in soil in which chlorothalonil had been incorporated showed reduction in longevity of about 50% compared to controls 52–84 days after the beginning of treatment. The amount of chlorothalonil added was equivalent to 5 times the recommended application rate at 0.9 g in 4700 cm³ of soil, and reproduction was virtually eliminated (Roark & Dale, 1979).

9.1.3.3 Earwigs and honey-bees

Earwigs (*Labidura riparia*) were exposed to chlorothalonil in three ways: a) on glass; b) as a residue on peanut foliage; and c) as a residue on a food source (i.e. 7-day-old armyworms). In the first treatment, chlorothalonil at a rate of 0.72 kg/ha produced up to 20% mortality in 24 h and up to 30% in 48 h. In the second experiment, chlorothalonil was applied, at the same rate, to peanut foliage which was then used for the test. There was a 10% mortality within 24 h and 20% in 48 h on 4-day-old residues and no mortality on 8-day-old residues. In the food source experiment there was 25-30% mortality within 24 h of the larvae being consumed and up to 55% mortality within 48 h (DeRivero & Poe, 1981).

Atkins et al. (1975) found no contact toxicity of chlorothalonil (11 µg/bee), and classified it as relatively non-toxic to honey-bees. The oral LD₅₀ for chlorothalonil in 20% sucrose solution for honey-bees was > 0.2 µg/bee and the contact LD₅₀ > 65 µg/bee (Davies, 1986).

9.1.3.4 Birds

The following toxicity values have been reported for birds, but without descriptions of the experimental methodology. The acute oral LD₅₀ in the mallard duck was reported to be > 4640 mg/kg. The 8-day dietary LC₅₀ in the same species and in the bobwhite quail (*Colinus virginianus*) was given as > 10 000 mg/kg diet in each case (SDS Biotech Corporation, 1981a,b). Dietary 8-day LC₅₀ values were also reported as > 21 500 mg/kg diet for the mallard duck and 5200 mg/kg diet for the bobwhite quail (Worthing, 1991).

Shults et al. (1988a) evaluated the effect of chlorothalonil on reproduction in the bobwhite quail. Four groups (16 pairs per group) of quail were administered chlorothalonil in the diet at levels of 1000, 5000 and 10 000 mg/kg for a period of 21 weeks. Quail were fed the amended diet for 11 weeks prior to egg laying and for the duration of the egg laying period. Adult and offspring were examined for body weight, general health, adult food consumption, egg production, eggshell thickness, embryo viability, hatching success, survivability of offspring and gross pathology. At a dietary concentration of 10 000 mg/kg, the birds experienced reproductive impairment, which included mortality, general health, body weight, food consumption, gross pathology and other

reproductive end-points. Hatching survival was also affected at the highest dosage. General health and reproductive parameters were also affected at 5000 mg/kg. Other effects observed at this dose level included decreased body weight gain and survivability of offspring. The lowest test dosage showed no apparent effects on either adult quail or offspring. A no-observed-effect concentration (NOEC) of 1000 mg/kg diet was established for chlorothalonil regarding reproductive effects.

In a separate but similar reproductive study with mallard ducks (*Anas platyrhynchos*), Shults et al. (1988b) reported that 10 000 mg/kg diet reduced egg production and the percentage of hatchlings per incubated egg. No reproductive impairments were observed for ducks dosed at 1000 or 5000 mg/kg. Shults et al. (1988b) reported no effect on eggshell thickness at these chlorothalonil concentrations. The NOEC for reproductive effects in mallard duck was 5000 mg/kg diet.

9.2 Field observations

9.2.1 Soil microorganisms

Smiley & Craven (1979) applied chlorothalonil (as Daconil 2787) at the normal rate (actual dose not given) every 21 days between April and September (9 applications for 3 consecutive years) to an experimental plot of Sward Kentucky blue grass (*Poa pratensis*). Treated plots were 1 x 5 m and were replicated. Samples of soil cores 2.54 cm in diameter and 3 cm deep were taken (n = 5) from each replicate plot. The cores included thatch and were chopped and mixed well, and 10 g was suspended in sterile distilled water. A dilution series was plated out to estimate bacterial, actinomycete and fungal populations. None of the organisms were affected by chlorothalonil. The fungicide did not affect numbers of *Nitrosomonas* or *Nitrobacter* bacteria and had no effect on the disappearance of added ammonium by nitrification. Treatments alternating different fungicides had a greater effect than one fungicide alone.

9.2.2 Plants

Many crop plants are tolerant of chlorothalonil and do not suffer any phytotoxicity when sprays of chlorothalonil formulations are applied according to recommended practices. However, a few phytotoxic effects have been observed with certain species as a direct result of chlorothalonil applications and

also as a result of physiologically incompatible mixtures of chlorothalonil formulations with additives or other pesticides.

Chlorothalonil applied at a rate of 2.52 kg/ha did not affect yields of tomato plants grown in field conditions. In addition, chlorothalonil did not enhance metribuzin damage when the two compounds were used in combination on tomato plants. These results are contrary to those obtained in laboratory studies (section 9.1.3) and indicate that under field conditions phytotoxic reactions between chlorothalonil and metribuzin are unlikely to occur (Stephenson et al., 1980).

Clear results were not obtained concerning the effect of chlorothalonil on perennial rye grass (*Lolium perenne*) when it was sprayed regularly for the control of leaf-spotting disease. In the first year of treatment the grass yield was increased by 15% at the third harvest, but at the following harvest, when disease incidence was greater, the yield was not increased. The authors concluded that chlorothalonil did not have a direct stimulatory effect on grass growth (Lam & Lewis, 1983).

Chlorothalonil was used to treat onion beds at a rate of 2.34 litres formulation/ha with 10 weekly applications. The onions (two short-day cultivars) were harvested either 146 or 167 days after planting. Chlorothalonil reduced the weight and size of marketable bulbs by 44 and 32%, respectively, but did not influence the maturation rate. Alternate application of chlorothalonil and mancozeb reduced these values by 27 and 33%, respectively. The disease incidence was nil in all treatments (Stoffella & Sonoda, 1982).

10. PREVIOUS EVALUATIONS BY INTERNATIONAL BODIES

The Joint FAO/WHO Meeting on Pesticide Residues (JMPR) discussed and evaluated chlorothalonil at its meetings in 1974, 1977, 1978, 1979, 1981, 1983, 1985, 1987, 1990 and 1992 (FAO/WHO, 1975, 1978, 1979, 1980, 1982, 1985, 1986a,b, 1988, 1990a,b, 1993b). In 1990 an acceptable daily intake (ADI) of 0-0.03 mg/kg body weight was established. This ADI was confirmed in 1992, based on the NOAEL of 3 mg/kg body weight/day established in the two-year dog study (FAO/WHO, 1993a,c).

The Joint FAO/WHO Codex Alimentarius Commission has established maximum residue limits (MRLs) for chlorothalonil in various commodities (FAO/WHO, 1993c).

WHO has classified chlorothalonil as a technical product unlikely to present an acute hazard in normal use (WHO, 1992).

On the basis of data available at the time, the International Agency for Research on Cancer evaluated chlorothalonil as showing limited evidence of carcinogenicity in animal studies and categorized it as an agent not classifiable as to its carcinogenicity to humans (IARC, 1987).

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RESUME

1. Identité, propriétés physiques et chimiques et méthodes d'analyse

Le chlorothalonil est un solide cristallin incolore et inodore, dont le point de fusion est de 250 °C et la tension de vapeur de $7,63 \times 10^{-6}$ Pa ($5,72 \times 10^{-7}$ mmHg) à 25 °C. Il est peu soluble dans l'eau (0,6-1,2 mg/litre à 25 °C) et son coefficient de partage entre l'octanol et l'eau ($\log K_{ow}$) est de 2,882. Dans l'eau, il s'hydrolyse lentement à pH 9 mais il est stable à pH ≤ 7 (à 25 °C).

La méthode d'analyse la plus courante, après extraction et purification, est la chromatographie gaz-liquide avec détection par capture d'électrons.

2. Sources d'exposition humaine et environnementale

Le chlorothalonil est produit depuis 1969 à des fins commerciales, soit par chloration de l'isophtalonitrile, soit en traitant le tétrachlorisophtaloylamide par l'oxychlorure de phosphore. C'est un fongicide à large spectre utilisé non seulement en agriculture, mais aussi pour traiter le gazon et les plantes ornementales. On l'utilise pour protéger les fruits à pépins et à noyaux, les agrumes, les groseilles, les baies, les bananes, les tomates, les légumes verts, le café, les arachides, les pommes de terre, les oignons et les céréales. En outre, il entre dans la composition de certains produits pour la conservation du bois et de certaines peintures.

Il existe en trois formulations principales, un concentré pour suspensions, des granulés dispersables dans l'eau et une poudre mouillable. Ces produits sont facilement dilués dans l'eau et épandus par pulvérisations au sol ou aériennes. La dose d'emploi est habituellement de 1,2 à 2,5 kg de matière active par hectare pour le traitement des haricots, des céleris et des oignons. L'exposition humaine a lieu principalement pendant la préparation et l'épandage du produit ou lors de l'ingestion de résidus présents dans certaines denrées alimentaires (voir section 1.1.4).

3. Transport, distribution et transformation dans l'environnement

Le chlorothalonil s'élimine des milieux aqueux par une forte adsorption sur les particules en suspension. La modélisation des

données disponibles montre qu'il ne migre pratiquement pas vers les sédiments du fond. Il est possible qu'il subisse une biodégradation enzymatique dans les eaux naturelles. Dans le sol, il est rapidement dégradé, cette dégradation pouvant également avoir lieu dans l'eau avec production du métabolite hydroxylé en position 4, c'est-à-dire le 4-hydroxy-2,5,6-trichlorisophtalonitrile. La demi-vie de dissipation du métabolite 4-hydroxy dans le sol est comprise entre 6 et 43 jours.

Une fois entré en contact avec une plante, le chlorothalonil ne migre pas vers d'autres zones du végétal. Les végétaux ne le métabolisent que dans une proportion limitée et le métabolite 4-hydroxy constitue en général moins de 5% du résidu.

Chez les poissons, la métabolisation du chlorothalonil comporte une conjugaison avec le glutathion, qui aboutit à des produits d'excrétion plus polaires. Elle s'effectue sous l'action de la glutathion-S-transférase. L'excrétion du composé sous forme de conjugué avec le glutathion est corroborée par le fait que, chez la truite arc-en-ciel, on trouve une forte concentration de marqueur dans la vésicule biliaire et dans la bile après exposition à du ¹⁴C-chlorothalonil. Une fois les poissons replacés en eau propre, on a constaté une chute rapide de la concentration du marqueur qui s'était accumulé dans la vésicule biliaire et les autres organes.

Le chlorothalonil ne s'accumule pas chez les organismes aquatiques.

4. Concentrations dans l'environnement et exposition humaine

Lors d'une étude sur des cultures de pommes de terre, on a pulvérisé du chlorothalonil sur un petit cours d'eau. Après prélèvement et analyse de l'eau en aval du secteur traité, on a constaté que le composé disparaissait rapidement (par ex. la concentration passait de 450 µg/litre 30 minutes après le traitement à 2-6 µg/litre 12 h après le traitement). Les traitements de routine effectués sur des cultures irriguées de plein champ, comme les pommes de terre ou l'orge, n'ont donné lieu qu'à de faibles concentrations de chlorothalonil (0,04-3,6 µg/litre), comme l'ont montré un certain nombre d'analyses pratiquées sur de l'eau prélevée à quelques occasions dans des drains en grès vernissé.

Les résidus qui subsistent sur les récoltes sont essentiellement constitués par le chlorothalonil lui-même. Leur concentration est

fonction de la dose d'emploi, du temps écoulé depuis le dernier épandage et la dernière récolte ainsi que du type de culture. A partir des nombreux essais effectués sous contrôle un peu partout dans le monde et dont les résultats ont été communiqués à la FAO et à l'OMS, il est possible de déterminer les concentrations de résidus présentes au moment de la récolte. En ce qui concerne les produits laitiers, il est vraisemblable que les résidus sont soit indétectables, soit très faibles. Dans le lait de vaches laitières qui avaient reçu pendant 30 jours du chlorothalonil mêlé à leur nourriture (jusqu'à 250 mg/kg), on n'a pas trouvé trace du composé, mais celui-ci était présent dans les tissus à très faible concentration.

Des analyses pratiquées dans plusieurs pays, soit sur la ration totale, soit sur tel ou tel aliment, ont montré, à l'occasions d'enquêtes par sondage, que le chlorothalonil n'était présent qu'en quantités indétectables ou du moins très faibles. Les diverses préparations que subissent les produits alimentaires, comme le pelage, le lavage et la cuisine en général contribuent d'ailleurs à abaisser encore leur teneur en résidus.

5. Cinétique et métabolisme chez les animaux de laboratoire

Chez des rats qui en recevaient par voie orale des doses allant jusqu'à 50 mg/kg de poids corporel, le composé a été absorbé à hauteur d'environ 30% en l'espace de 48 h. A plus forte dose, l'absorption est moindre, ce qui est le signe d'un processus de saturation. Après administration de ¹⁴C-chlorothalonil par voie orale, on a observé une répartition tissulaire et sanguine de la radioactivité en 2 h. C'est au niveau des reins, du foie et du sang - dans cet ordre - qu'ont été relevées les concentrations les plus importantes. Au bout de 24 h, on a mesuré, au niveau des reins, une concentration égale à 0.3% d'une dose initiale de 5 mg/kg de poids corporel.

La majeure partie d'une dose administrée par voie orale à des rats a été retrouvée dans leurs matières fécales (> 82% en l'espace de 48-72 h, quelle que soit la dose initiale). L'excrétion biliaire est rapide, culminant au bout de 2 h après ingestion d'une dose de 5 mg/kg de poids corporel et la saturation est atteinte à partir de 50 mg/kg de poids corporel. Chez le rat, la dose est excrétée à hauteur de 5-10% par la voie urinaire. Chez le chien et le singe, la principale voie d'excrétion est la voie fécale, la voie urinaire étant moins importante que chez le rat (< 4%).

Les études métaboliques menées sur des rats montrent que le chlorothalonil est conjugué avec le glutathion dans le foie ainsi que dans les voies digestives. Certains de ces conjugués peuvent être absorbés dans l'intestin et parvenir jusqu'aux reins où ils sont transformés par la β -lyase du cytosol en analogues thioliques, excrétés ensuite par la voie urinaire. Lorsqu'on administre du chlorothalonil à des rats axéniques, les métabolites thioliques apparaissent dans l'urine en quantité bien moindre que chez des rats normaux, ce qui indique que la flore intestinale intervient dans le métabolisme de ce composé. Des chiens et des singes à qui on administre du chlorothalonil par voie orale, n'excrètent que peu ou pas de métabolites thioliques dans leurs urines.

Après application cutanée de ^{14}C -chlorothalonil à des rats, environ 28% de la dose ont été absorbés en 120 h. On a retrouvé environ 18% de la dose dans les matières fécales et 6% dans les urines au bout de 120 h.

6. Effets sur les mammifères de laboratoire et les systèmes d'épreuve *in vitro*

Chez le rat et le lapin, la toxicité aiguë du chlorothalonil est faible, que ce soit par voie orale ou en applications cutanée ($\text{DL}_{50} > 10\ 000$ mg/kg de poids corporel). Du chlorothalonil broyé au mortier (D médian des particules égal à 5-8 μm) s'est révélé très toxique pour des rats lors d'une étude toxicologique par inhalation, avec une CL_{50} à 4 h de 0,1 mg/litre.

Le chlorothalonil est irritant pour la peau et les yeux chez le lapin. Les études de sensibilisation cutanée effectuées sur des cobayes n'ont pas été concluantes.

Chez le rat, les principaux effets de doses orales répétées s'exercent au niveau des reins et de l'estomac. Pendant 13 semaines, on fait ingérer quotidiennement à des rats, répartis en groupes de 25 animaux de chaque sexe, du chlorothalonil mêlé à leur nourriture aux doses de 0, 1,5, 3, 10, ou 40 mg/kg de poids corporel. Après une période de récupération de 13 semaines, les rats ont été sacrifiés et l'on a observé une hyperplasie et une hyperkératose au niveau de la portion cardiaque de l'estomac aux doses de 10 et 40 mg/kg; ces lésions ont regressé lorsque le traitement a cessé. A la dose de 40 mg/kg, on notait chez les mâles une augmentation de l'incidence des hyperplasies épithéliales au niveau des tubules proximaux du rein au bout des 13 semaines de traitement ainsi qu'après la période de récupération. La dose

sans effet observable a été évaluée à 3 mg/kg de poids corporel par jour, le critère retenu étant l'absence de lésions au niveau de la portion cardiaque de l'estomac. Les lésions intéressant cette partie de l'estomac ainsi que les reins sont apparues rapidement, à savoir en 4 à 7 jours chez les mâles lorsqu'on a porté la dose alimentaire quotidienne à 175 mg/kg de poids corporel.

Lors d'une étude de 13 semaines sur des souris (0, 7,5, 15, 50, 275, ou 750 mg/kg en mélange à la nourriture), on a constaté une incidence accrue des hyperplasies et des hyperkératoses de l'épithélium pavimenteux au niveau de la portion cardiaque de l'estomac. Ces lésions ont été observées chez les mâles comme chez les femelles à partir de 50 mg/kg de nourriture. En se basant sur la présence ou l'absence de ces lésions, on a évalué à 15 mg/kg de nourriture la dose de chlorothalonil sans effet observable, soit l'équivalent quotidien de 3 mg/kg de poids corporel.

Une étude de 16 semaines sur des chiens aux doses alimentaires de 0, 250, 500, ou 750 mg/kg n'a pas révélé de d'effets qui soient imputables au traitement.

Les lésions observées au niveau des reins et de la portion cardiaque de l'estomac ont fait l'objet, pendant 2 ans, d'études plus approfondies sur des souris et des chiens. Une autre étude, portant cette fois sur des rats (aux doses quotidiennes de 0, 1,8, 3,8, 15 ou 175 mg/kg de poids corporel), a permis de caractériser histologiquement les effets observés: il s'agissait d'une part, d'une augmentation de l'incidence des hyperplasies, des hyperkératoses, des ulcérations et des abrasions de l'épithélium pavimenteux au niveau de la portion cardiaque de l'estomac et, d'autre part, de la présence d'une hyperplasie au niveau des tubules contournés proximaux du rein. Ces anomalies ont été observées à partir de 3,8 mg/kg. A la dose de 175 mg/kg, on notait un accroissement sensible de l'incidence des tumeurs rénales (adénomes et carcinomes) et des tumeurs intéressant la portion cardiaque de l'estomac (papillomes et carcinomes). On est fondé à penser que l'incidence des tumeurs rénales était augmentée chez les mâles à partir de la dose de 15 mg/kg, de même que celle des tumeurs gastriques chez les deux sexes aux doses de 3,8 mg/kg et de 15 mg/kg. La dose sans effets néoplasiques observables a donc été prise égale à 1,8 mg/kg, en prenant comme critère l' incidence des tumeurs au niveau de la portion cardiaque de l'estomac. Une autre étude de 2 ans, au cours de laquelle des doses plus élevées ont été utilisées, a confirmé le pouvoir cancérogène du chlorothalonil, tant au niveau du rein que de la portion cardiaque de l'estomac.

Une étude sur des souris (doses de 0, 15, 40, 175, ou 750 mg/kg de nourriture) a révélé une augmentation de l'incidence des hyperplasies au niveau des tubules rénaux à partir de 175 mg/kg, le même phénomène étant observé à partir de 40 mg/kg au niveau de la portion cardiaque de l'estomac, avec en outre une hyperkératose. A la dose de 750 mg/kg, il y avait une légère augmentation des tumeurs spinocellulaires au niveau de la portion cardiaque de l'estomac. On en a donc conclu que les doses sans effets néoplasiques ou non néoplasiques étaient respectivement égales à 175 et 15 mg/kg de nourriture (soit l'équivalent quotidien de 17,5 et 1,6 mg/kg de poids corporel, respectivement). Ces effets constatés chez la souris sont corroborés par les résultats d'une autre étude avec des doses plus élevées, mais une troisième investigation portant sur des souris B6C3F₁ n'a pas mis en évidence d'effets cancérogènes à dose élevée.

Lors d'une étude de 2 ans sur des chiens (60 et 120 mg/kg de nourriture), aucun effet attribuable au chlorothalonil n'a été observé. On en conclut que la dose sans effet observable était de 120 mg/kg de nourriture (soit l'équivalent quotidien de 3 mg/kg de poids corporel).

Plusieurs épreuves de mutagénicité *in vivo* et *in vitro* se sont révélées négatives, mais il y en a tout de même eu quelques unes de positives.

Les dérivés monothio, dithio, trithio, dicystéinyl, tricystéinyl et monoglutathionyl du chlorothalonil, qui sont potentiellement néphrotoxiques, se sont révélés négatifs dans l'épreuve d'Ames.

Le chlorothalonil ne s'est pas montré tératogène pour le rat ou le lapin à des doses quotidiennes atteignant respectivement 400 et 50 mg/kg de poids corporel. Lors d'une étude sur deux générations de rats, on n'a pas constaté d'effets sur l'accouplement, la fécondité ou la gestation jusqu'à des doses atteignant 1500 mg/kg de nourriture.

La toxicité aiguë par voie orale du métabolite 4-hydroxy est supérieure à celle du chlorothalonil lui-même (DL_{50} aiguë par voie orale égale à 332 mg/kg de poids corporel contre > 10 000 mg/kg de poids corporel). Plusieurs études ont été entreprises pour caractériser le profil toxicologique de ce métabolite et établir les doses sans effets observables.

7. Effets sur l'homme

On a signalé des cas de dermatite de contact parmi des personnes employées à la fabrication de chlorothalonil, chez des agriculteurs et des horticulteurs. D'autres cas de dermatite siégeant au niveau des mains et de la face ont été observés chez des personnes qui utilisaient des produits de protection du bois à base de chlorothalonil.

8. Effets sur les autres êtres vivants au laboratoire et dans leur milieu naturel

Le chlorothalonil est extrêmement toxique pour les poissons et les invertébrés aquatiques, comme le montrent un certain nombre d'études en laboratoire, avec des valeurs de la CL_{50} inférieures à 0,5 mg/litre. La concentration maximale acceptable de produit toxique (MATC) s'est révélée être égale à 35 µg/litre lors d'une étude sur deux générations de daphnies.

A quelques exceptions près, d'ailleurs sans grande importance, le chlorothalonil n'est pas phytotoxique.

La CL_{50} d'un concentré pour suspension (500 g de chlorothalonil par litre) répandu sur un sol artificiel pour lombrics, a été évaluée à > 1000 mg/kg de terre (14 jours). On a observé une surmortalité parmi des scolopendres qui s'étaient trouvés en contact avec des résidus de chlorothalonil présents sur des feuilles d'arachide ou s'en étaient nourris au laboratoire; il n'y a pas eu d'autre indice d'une action insecticide.

Le chlorothalonil est peu toxique pour les oiseaux, comme le montre la valeur de la DL_{50} aiguë par voie orale chez le colvert (4640 mg/kg). Aucun effet important sur la reproduction n'a été signalé.

D'après une étude effectuée sur le terrain, la toxicité du chlorothalonil pour les organismes aquatiques est moindre que ne le font craindre les expériences de laboratoire; ce résultat est en accord avec les propriétés physico-chimiques de ce composé. On a tout de même enregistré une mortalité chez des espèces exposées expérimentalement sur le terrain. En revanche, on n'a pas signalé d'accidents écologiques ayant entraîné une mortalité. Malgré la faible persistance du chlorothalonil dans les divers compartiments du milieu, il faut tout de même s'attendre à une certaine mortalité. En pareil cas, il sera difficile d'établir un lien de cause à effet

étant donné que les résidus de chlorothalonil ne subsistent pas suffisamment longtemps pour que l'on puisse identifier le composé.

RESUMEN

1. Identidad, propiedades físicas y químicas, y métodos analíticos

El clorotalonilo es un sólido cristalino inodoro e incoloro con un punto de fusión de 250 °C y una presión de vapor de $7,63 \times 10^{-5}$ Pa ($5,72 \times 10^{-7}$ mmHg) a 25 °C. Es poco soluble en agua (0,6 a 1,2 mg/litro a 25 °C) y tiene un coeficiente de partición octanol/agua ($\log K_{oa}$) de 2,882. Se hidroliza lentamente en agua con un pH de 9, pero es estable a un pH de 7 o inferior (a 25 °C).

El método analítico más corriente, después de la extracción y depuración de las muestras, es la cromatografía gas-líquido empleando un detector de captura de electrones.

2. Fuentes de exposición del ser humano y del medio ambiente

El clorotalonilo se viene produciendo a escala comercial desde 1969 por cloración del isoftalonitrilo o mediante el tratamiento de la amida tetracloroisofalolil con oxiclورو de fósforo. Es un fungicida con amplio espectro de actividad empleado principalmente en la agricultura pero también en el césped, los pastos y las plantas ornamentales. Los cultivos protegidos incluyen frutas de pepitas y de hueso, cítricos, grosellas, fresas, bananas, tomates, verduras, café, cacahuete, patatas, cebollas y cereales. Se emplea también en sustancias protectoras de la madera y en pinturas.

Las tres formulaciones principales son una suspensión concentrada, un gránulo hidrodispersible y un polvo humectable. Se disuelven fácilmente en agua y se aplican empleando sistemas de pulverización en los suelos o rociado aéreo. Las tasas típicas del ingrediente activo oscilan entre 1,2 y 2,5 kg/ha en el caso de cultivos tales como frijoles, apio y cebollas. Las principales fuentes de exposición del ser humano son la preparación y aplicación de los productos, así como la ingestión de residuos de las cosechas en los alimentos (véase la sección 1.1.4).

3. Transporte, distribución y transformación en el medio ambiente

El clorotalonilo se elimina de los medios acuosos mediante intensa adsorción en las materias en suspensión. Los datos de los

modelos parecen indicar poca, o ninguna, partición detectable en el sedimento del fondo. Puede producirse biodegradación en aguas naturales, con la participación de procesos enzimáticos. El clorotalonilo se degrada rápidamente en el suelo, y puede haber degradación en el agua con la producción del metabolito 4-hidroxi-2,5,6-tricloroisoftalonitrilo. La semivida para la disipación en el suelo de ese metabolito varía entre 6 y 43 días.

El clorotalonilo no se transloca del punto de aplicación a otras partes de la planta. Su metabolización en las plantas es limitada y, por lo general, ese metabolito representa < 5% del residuo.

En cuanto a los peces, el clorotalonilo se metaboliza mediante la conjugación con glutatión para producir productos de degradación más polares. En esa conversión interviene la enzima glutatión-S-transferasa. Las elevadas concentraciones del radioisótopo marcador detectadas en la vesícula biliar y la bilis después de la exposición de la trucha arco iris al ¹⁴C-clorotalonilo son compatibles con la excreción del compuesto en forma de conjugados de glutatión. Las concentraciones de los radioisótopos de trazado que se acumulan en la vesícula biliar y otros órganos disminuyen rápidamente al colocar los peces en agua no contaminada.

El clorotalonilo no experimenta bioacumulación en los organismos acuáticos.

4. Niveles ambientales y exposición humana

En un estudio de un cultivo de patatas, se procedió a rociar un pequeño arroyo con clorotalonilo. Muestreos y análisis posteriores del agua río abajo demostraron la rápida desaparición del clorotalonilo (las concentraciones eran de 450 µg/litro a los 30 minutos después del rociado, y oscilaban entre 2 y 6 µg/litro a las 12 h después del rociado). El rociado sistemático de los cultivos irrigados como, por ejemplo, patatas y cebada, estuvo acompañado de bajas concentraciones de clorotalonilo (0,04 a 3,6 µg/litro) en el agua de los tubos de drenaje en un pequeño número de muestras.

Los residuos de las cosechas están compuestos principalmente de clorotalonilo propiamente dicho. Las concentraciones residuales dependen del nivel aplicado, del tiempo transcurrido entre la última aplicación y la cosecha, y del tipo de cosecha. Los niveles residuales en la cosecha pueden deducirse de los numerosos

ensayos supervisados realizados con muchas cosechas en todo el mundo y comunicados a la FAO y la OMS. Se prevé que los residuos de clorotalonilo en los productos lácteos serán imposibles de detectar, o las concentraciones muy bajas. En un estudio con vacas lecheras alimentadas durante 30 días con pienso al que se habían añadido elevadas concentraciones (hasta 250 mg/kg) de clorotalonilo no se observó ningún residuo detectable en la leche y sólo niveles muy bajos en los tejidos.

Los análisis del régimen alimenticio total y de alimentos específicos realizados en varios países han revelado concentraciones no detectables, o muy bajas, de clorotalonilo en los estudios por muestreo. Los procesos de preparación tales como el lavado, el pelado y la cocción permiten reducir aún más los niveles residuales en los alimentos.

5. Cinética y metabolismo en animales de laboratorio

En estudios con ratas a las que se administraron dosis de hasta 50 mg/kg de peso corporal, se observó que un 30% de la dosis oral de clorotalonilo se absorbía dentro de las 48 horas. La absorción es inferior con posologías más elevadas, lo que indica un proceso de saturación. Cuando se administra oralmente ¹⁴C-clorotalonilo, la radioactividad se distribuye en la sangre y los tejidos en menos de dos horas. Las mayores concentraciones se encuentran en el riñón, el hígado y la sangre, en ese orden. Con una posología de 5 mg/kg de peso corporal, la concentración en los riñones es 0,3% a las 24 horas.

Por lo que respecta a las ratas, la mayor parte de la dosis oral de clorotalonilo se encuentra en las heces (> 82% dentro de las 48 a 72 horas, independientemente de la dosis). Con una dosis oral de 5 mg/kg de peso corporal, la excreción biliar es rápida, alcanzando su valor máximo dentro de las 2 horas, ocurriendo saturación con posologías de 50 mg/kg de peso corporal y superiores. En el caso de las ratas, la excreción urinaria representa entre un 5 y 10% de la dosis. En perros y monos, la excreción fecal es la vía principal, pero la excreción urinaria (< 4%) es inferior a la observada en las ratas.

Estudios metabólicos llevados a cabo con ratas indican que el clorotalonilo se conjuga con el glutatión tanto en el hígado como en el tracto gastrointestinal. Algunos de los conjugados del glutatión pueden ser absorbidos en el intestino y transportados a los riñones donde son convertidos por la β -liasa citosólica en

análogos del tiol que se excretan por la orina. Cuando se dan dosis de clorotalonilo a ratas axénicas, se observan en la orina metabolitos del tiol en cantidades muy inferiores a las registradas en ratas normales, lo que indica que la microflora intestinal interviene en el metabolismo del clorotalonilo. En el caso de los perros o monos que reciben dosis orales de clorotalonilo, la excreción de derivados del tiol por la orina no es detectable, o es muy baja.

Cuando se aplicó ¹⁴C-clorotalonilo a la piel de la rata, un 28% de la dosis fue absorbida en menos de 120 horas. Se observaron concentraciones del 18% de la dosis en las heces y del 6% en la orina al cabo de 120 horas.

6. Efectos en mamíferos de laboratorio y sistemas de pruebas *in vitro*

El clorotalonilo tiene baja toxicidad oral y cutánea en ratas y conejos, respectivamente (los valores agudos orales y cutáneos de la DL₅₀ son > 10 000 mg/kg de peso corporal). Por lo que respecta a las ratas, en un estudio de inhalación se observó que el clorotalonilo técnico pulverizado (MMAD 5 a 8 μm) presentaba elevada toxicidad, con una CL₅₀ de 0,1 mg/litro a las 4 h.

El clorotalonilo es un irritante de la piel y los ojos en el conejo. Los estudios de sensibilización cutánea en el conejillo de indias no arrojaron resultados concluyentes.

En el caso de las ratas, los efectos principales de dosis orales repetidas de clorotalonilo se observan en el estómago y los riñones. En un estudio con grupos de 25 ratas, en que se separaron los sexos, se emplearon posologías de 0, 1,5, 3, 10 ó 40 mg/kg de peso corporal por día en la dieta durante 13 semanas, lo que estuvo seguido de un periodo de recuperación de 13 semanas. Se observó mayor frecuencia de hiperplasia y hiperketarosis del preestómago con las posologías de 10 y 40 mg/kg; los efectos desaparecieron cuando cesó el tratamiento. Con una concentración de 40 mg/kg, se registró mayor incidencia de hiperplasia del epitelio tubular proximal del riñón en los machos a las 13 semanas y después del periodo de recuperación. El nivel sin efecto observado fue de 3 mg/kg de peso corporal por día en base a la ausencia de lesiones en el preestómago. Se ha demostrado que los cambios observados en el preestómago y los riñones son de rápida aparición, presentándose las lesiones dentro de un periodo de 4 a 7 días en el caso de los machos, cuyo régimen alimenticio incluía una concentración de 175 mg/kg de peso corporal al día.

En un estudio de 13 semanas de duración realizado con ratones (empleando dosis de 0, 7,5, 15, 50, 275 ó 750 mg/kg en el alimento), se observó mayor incidencia de hiperplasia e hiperkeratosis de las células epiteliales escamosas del preestómago en el caso de los machos y las hembras cuando se emplearon posologías de 50 mg/kg y superiores. Atendiendo a esos cambios, el nivel sin efecto observado fue de 15 mg/kg de clorotalonilo en la dieta, lo que equivale a 3 mg/kg de peso corporal por día.

Un estudio de 16 semanas de duración con perros cuyo alimento contenía concentraciones de 0, 250, 500 ó 750 mg/kg no reveló cambios relacionados con el tratamiento.

Se llevaron a cabo investigaciones adicionales de las lesiones en el preestómago y los riñones, llevándose a cabo estudios con ratas, ratones y perros durante un periodo de 2 años. En un estudio realizado con ratas (empleando dosis de 0, 1,8, 3,8, 15 ó 175 mg/kg de peso corporal al día), los efectos estuvieron caracterizados histológicamente por una mayor incidencia e intensidad de hiperplasia, hiperkeratosis, y úlceras y erosiones de la mucosa escamosa del preestómago, y por hiperplasia del epitelio de los túbulos contorneados proximales de los riñones con posologías de 3,8 mg/kg y superiores. Por lo que respecta a los efectos no neoplásicos, el nivel sin efecto observado fue, por lo tanto, de 1,8 mg/kg. La incidencia de tumores renales (adenomas y carcinomas) y de tumores del preestómago (papilomas y carcinomas) fue considerablemente superior, alcanzando 175 mg/kg. Hubo pruebas de mayor incidencia de tumores renales en los machos con dosis de 15 mg/kg, así como de tumores estomacales en los machos y las hembras con dosis de 3,8 y 15 mg/kg. Por lo que respecta a los efectos neoplásicos, el nivel sin efecto observado fue, por lo tanto, de 1,8 mg/kg de peso corporal por día sobre la base de los cambios en la incidencia de tumores en el preestómago. El riesgo carcinogénico del clorotalonilo en los riñones y preestómago de las ratas se vio corroborado por los resultados de otros estudios de 2 años de duración en que emplearon dosis más elevadas.

En un estudio con ratones (empleando dosis de 0, 15, 40, 175 ó 750 mg/kg en el alimento), se observó mayor incidencia de hiperplasia tubular renal con dosis de 175 mg/kg y superiores, así como de hiperplasia y hiperkeratosis del preestómago con concentraciones de 40 mg/kg y superiores. La incidencia de tumores escamosos del preestómago aumentó ligeramente con dosis de 750 mg/kg. Por consiguiente, por lo que respecta a los cambios

neoplásicos y no neoplásicos, los niveles sin efecto observado fueron de 175 y 15 mg/kg en la dieta (lo que equivale a 17,5 y 1,6 mg/kg de peso corporal por día, respectivamente). Otro estudio con posologías superiores corroboró esos efectos en el ratón, pero un estudio con ratones B6C3F₁, no señaló ningún riesgo carcinogénico con dosis elevadas.

En un estudio de 2 años de duración con perros (empleando 60 y 120 mg/kg en el alimento), no se detectó ningún efecto atribuible al clorotalonilo. Por lo tanto, el nivel sin efecto observado fue de 120 mg/kg en el alimento (lo que equivale a 3 mg/kg de peso corporal al día).

El clorotalonilo no resultó mutagénico en varias pruebas *in vitro* e *in vivo*, aunque fue positivo en un pequeño número de valoraciones.

Los derivados monotio, ditio, tritio, dicisteina, tricisteina y monoglutación del clorotalonilo, que son posibles sustancias nefrotóxicas, arrojaron resultados negativos en el análisis de Ames.

El clorotalonilo no resultó teratogénico en las ratas o conejos con dosis de hasta 400 y 50 mg/kg de peso corporal al día, respectivamente. En un estudio realizado con dos generaciones de ratas, los parámetros reproductivos tales como el apareamiento, la fertilidad y el período de gestación no se vieron afectados por el clorotalonilo con concentraciones de hasta 1500 mg/kg en la dieta.

La toxicidad oral aguda del metabolito 4-hidróxido es superior a la del clorotalonilo propiamente dicho (DL_{50} oral aguda de 332 mg/kg de peso corporal en comparación con > 10 000 mg/kg de peso corporal). Se han llevado a cabo varios estudios destinados a caracterizar el perfil toxicológico de ese metabolito y a establecer los niveles sin efecto observado.

7. Efectos en el ser humano

Se ha informado de dermatitis por contacto en el caso de personas que trabajan en la producción de clorotalonilo, así como en el de agricultores y hortelanos. Se tienen noticias también de trabajadores de fábricas de productos de la madera que han contraído dermatitis por contacto en las manos y el rostro cuando empleaban preservativos de la madera que contenían clorotalonilo.

8. Efectos en otros organismos en el laboratorio y sobre el terreno

El clorotalonilo es sumamente tóxico para los peces y los invertebrados acuáticos en los estudios de laboratorio, siendo los valores de CL_{50} inferiores a 0,5 mg/litro. En un estudio sobre reproducción realizado con dos generaciones de *Daphnia magna*, la concentración tóxica máxima aceptable (CTMA) fue de 35 μ g/litro.

Con pocas excepciones, el clorotalonilo no es fitotóxico.

En lombrices, la CL_{50} de una formulación de una suspensión concentrada (500 g de clorotalonilo/litro) en suelo artificial fue > 1000 mg/kg de suelo (14 días). Las tijeretas experimentaron mayor mortalidad cuando estaban en contacto con residuos de clorotalonilo en las hojas del cacahuete o lo ingerían en su fuente de alimentos en las pruebas de laboratorio; no hubo ningún otro indicio de efecto insecticida.

El clorotalonilo tiene poca toxicidad para las aves, habiéndose informado de una DL_{50} oral aguda de 4640 mg/kg de alimento en el pato real. No se informó de ningún efecto considerable sobre la reproducción.

Un estudio sobre el terreno de organismos acuáticos expuestos después de la aplicación de clorotalonilo parece indicar que la toxicidad es inferior a la que cabría esperar atendiendo a los estudios de laboratorio; esto es también compatible con las propiedades fisicoquímicas de los compuestos. Se observaron muertes en algunas especies expuestas experimentalmente en el campo. No se ha informado de incidentes de muertes en el medio ambiente. Sin embargo, a pesar del corto tiempo de presencia del clorotalonilo en el medio ambiente, cabría esperar que ocurran muertes. Resultaría difícil establecer vínculos entre las muertes y los compuestos ya que la persistencia de los residuos no sería suficientemente prolongada para poder identificar el clorotalonilo.

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