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Chlordane as undesirable substance in animal feed[1]

Scientific Panel on Contaminants in the Food Chain

(Question N° EFSA-Q-2005-181)

Adopted on 7 November 2007

PANEL MEMBERS

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SUMMARY

Chlordane was commercially introduced as a non-systemic (not taken up in the plant), contact and ingested insecticide in 1947. Technical chlordane is a mixture, which consists of at least 147 compounds and the composition varies with the manufacturing process. It contained 43 – 75 % *cis*- and *trans*-chlordane and lesser amounts of heptachlor, *cis*- and *trans*-nonachlor and chlordenes. After 1970, a more refined formulation containing >95 % of *cis*- and *trans*-chlordane was also produced. Chlordane was used for agricultural purposes, mainly for soil and seed treatment and wood protection, the latter being applied mostly in the USA. It has been banned for use in the European Union since 1981 and currently in most other countries worldwide. In the environment chlordane is relatively stable and can be transported over long ranges. Chlordane is included in the Stockholm convention on persistent organic pollutants (POPs)[2] and the United Nations Economic Commission for Europe (UNECE) Convention on long-range transboundary air pollution protocol on POPs (CLRTAP-POP). Because of their lipophilic properties and persistence in the environment, chlordane and related compounds are bioaccumulated and biomagnified along the food chain. Chlordane compounds show

[1] For citation purposes: Opinion of the Scientific Panel on Contaminants in the Food Chain on a request from the European Commission on chlordane as undesirable substance in animal feed, *The EFSA Journal* (2007)582, 1-52

[2] http://www.pops.int/documents/convtext/convtext_en.pdf

increasing persistency with increasing number of chlorine atoms. Chlordane shows moderate acute toxicity. Oxychlordane (a major metabolite of *cis*- and *trans*-chlordane) and nonachlor are more toxic than *cis*- and *trans*-chlordane. In mammals, the main target organs are the nervous system and the liver. Chlordane causes liver tumours in mice, probably via non-genotoxic mechanisms. Chlordane is classified by IARC as possibly carcinogenic to humans (group 2B). Chlordane is moderately to highly toxic to fish exposed via water but no toxicity data following exposure via fish feed have been identified for fish. In laying hens, chlordane at high doses compromised egg production. For dogs, a NOAEL of 0.075 mg/kg b.w. per day (3 mg/kg feed) was identified based on liver toxicity in a long-term study. No LOAEL or NOAEL in farm animal species or pets could be derived from the studies identified. Chlordane (expressed as the sum of *cis*- and *trans*-chlordane and oxychlordane, and sometimes *cis*- and *trans*-nonachlor) is not frequently found in feed commodities except for fish derived products. The concentrations found are in the low µg/kg range and thus well below that found to cause adverse effects in dogs. The metabolism and excretion of chlordane and related compounds vary greatly between species. Oxychlordane and *trans*-nonachlor are generally the major residues of chlordane compounds in animal tissues and animal products. The current human dietary exposure to chlordane is in the low ng/kg b.w. per day range, which is two to three orders of magnitude below the provisional tolerable daily intake of 500 ng/kg b.w. established by the WHO in 1995.

KEYWORDS: Chlordane, chlordene, oxychlordane, heptachlor, nonachlor, persistence, insecticide, animal feed, toxicity, analysis, occurrence, metabolism, bioaccumulation, animal health, carry-over, human exposure, human health.

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LIST OF ABBREVIATIONS

ASE	Accelerated solvent extraction
ATSDR	Agency for Toxic Substances and Disease Registry
B.w.	Body weight
CAS	Chemical Abstract Service
CEN	European Committee for Standardisation
CYP	Cytochrome P450
ECD	Electron capture detection
EI	Electron impact
EMRL	Extraneous maximum residue limits
FAO	Food and Agriculture Organization
FEFAC	European Feed Manufacturers Federation
GABA	Gamma-aminobutyric acid
GC	Gas chromatography
GPC	Gel permeation chromatography
HR	High resolution
IARC	International Agency on Research on Cancer
IPCS	International Programme on Chemical Safety
JMPR	Joint WHO/FAO meeting on pesticide residues
LC_{50}	Concentration that causes death among 50 % of treated animals
LD_{50}	Dose that causes death among 50 % of treated animals
LOAEL	Lowest observed adverse effect level
LOD	Limit of determination
LOQ	Limit of quantification
MAE	Microwave assisted extraction
ML	Maximum level
MRL	Maximum residue level
MS	Mass spectrometry
NCI	Negative chemical ionization
NOAEL	No observed adverse effect level
OJ	Official Journal of the European Union
PCB	Polychlorinated biphenyl
POP	Persistent organic pollutant
SCAN	Scientific Committee on Animal Nutrition
SFE	Supercritical fluid extraction
SPE	Solid phase extraction
WHO	World Health Organization

BACKGROUND AS PROVIDED BY THE REQUESTOR

1. General background

Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed[3] replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition[4].

The main modifications can be summarised as follows

- extension of the scope of the Directive to include the possibility of establishing maximum limits for undesirable substances in feed additives.
- deletion of the existing possibility to dilute contaminated feed materials instead of decontamination or destruction (introduction of the principle of non-dilution).
- deletion of the possibility for derogation of the maximum limits for particular local reasons.
- Introduction of the possibility of the establishment of an action threshold triggering an investigation to identify the source of contamination ("early warning system") and to take measures to reduce or eliminate the contamination ("pro-active approach").

In particular the introduction of the principle of non-dilution is an important and far- reaching measure. In order to protect public and animal health, it is important that the overall contamination of the food and feed chain is reduced to a level as low as reasonably achievable providing a high level of public health and animal health protection. The deletion of the possibility of dilution is a powerful means to stimulate all operators throughout the chain to apply the necessary prevention measures to avoid contamination as much as possible. The prohibition of dilution accompanied with the necessary control measures will effectively contribute to safer feed.

During the discussions in view of the adoption of Directive 2002/32/EC the Commission made the commitment to review the provisions laid down in Annex I on the basis of updated scientific risk assessments and taking into account the prohibition of any dilution of contaminated non-complying products intended for animal feed. The Commission has therefore requested the Scientific Committee on Animal Nutrition (SCAN) in March 2001 to

[3] OJ L140, 30.5.2002, p. 10
[4] OJ L 115, 4.5.1999, p. 32

provide these updated scientific risk assessments in order to enable the Commission to finalise this review as soon as possible (Question 121 on undesirable substances in feed)[5].

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003[6] provides a comprehensive overview on the possible risks for animal and public health as the consequence of the presence of undesirable substances in animal feed.

It was nevertheless acknowledged by SCAN itself and by the Standing Committee on the Food Chain and Animal Health that for several undesirable substances additional detailed risk assessments are necessary to enable a complete review of the provisions in the Annex.

2. Specific background

Chlordane has been used as a pesticide. Technical chlordane is not a single chemical, but is actually a complex mixture of many related compounds. It does not occur naturally in the environment.

The use of chlordane as a pesticide has been banned in the EU since 1981 by Council Directive 79/117/EEC of 21 December 1978[7] which prohibited the placing on the market and use of plant protection products containing certain substances.

EU legislation on maximum residue levels (MRLs) for pesticides is laid down in four Council Directives

- Council Directive 76/895/EEC of 23 November 1976 relating to the fixing of maximum levels for pesticide residues in and on fruit and vegetables[8]
- Council Directive 86/362/EEC of 24 July 1986 on the fixing of maximum residue levels for pesticide residues in and on cereals[9]
- Council Directive 86/363/EEC of 24 July 1986 on the fixing of maximum residue levels for pesticide residues in and on foodstuffs of animal origin[10]
- Council Directive 90/642/EEC of 27 November 1990 on the fixing of maximum residue levels for pesticide residues in and on certain products of plant origin, including fruits and vegetables[11].

[5] Summary record of the 135th SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu.int/comm/food/fs/sc/scan/out61_en.pdf)
[6] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, adopted on 20 February 2003, updated on 25 April 2003 (http://europa.eu.int/comm/food/fs/sc/scan/out126_bis_en.pdf)
[7] OJ L 33, 8.2.1979, p. 36
[8] OJ L 340, 9.12.1976, p.26
[9] OJ L 221, 7.8.1986, p. 37
[10] OJ L 221, 7.8.1986, p. 43
[11] OJ L 350, 14.12.1990, p. 71

Until 1997, MRLs were fixed only for raw commodities. Council Directive 1997/41/EC of 25 June 1997[12] amending the above mentioned Directives, provided for a system applicable from 1 January 1999 to set MRLs in processed products and composite foodstuffs, based on the MRLs fixed for the raw agricultural products. MRLs for processed products and composite foodstuffs are calculated on the basis of the MRL set for the agricultural commodity by application of an appropriate dilution or concentration factor and for composite foodstuffs MRLs are calculated taking into account the relative concentrations of the ingredients in the composite foodstuffs. As the consequence of the coming into force of Directive 1997/41/EC, the pesticide residue legislation applies also to animal feedingstuffs since 1 January 1999.

Regulation (EC) No 396/2005 of the European Parliament and of the Council of 23 February 2005 on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC[13] will replace the abovementioned Directives once it is applicable.

However some problems have currently been observed in implementing the pesticide residue legislation to animal feedingstuffs. The following problems have already been identified:

- compound feed is composed of a relatively high number of ingredients, of which several are processed products (by-products). It is not obvious to know what MRL is applicable to such compound feed as it involves many calculations and uncertainties and "unknowns" (processing factors),
- pesticide residue legislation does not yet cover products of marine origin which are regularly used in animal feed (no direct application),
- pesticide residue legislation does not yet cover products typically for animal feed (no food use) such as pastures, roughages, forages, fish oil and fish meal.

Chlordane (sum of *cis*- and *trans*-isomers and of oxychlordane, expressed as chlordane) is listed in the Annex to Directive 2002/32/EC.

In the following table the provisions on the maximum levels for chlordane in the Annex to Directive 2002/32/EC are compared with the provisions foreseen in the pesticide legislation.

[12] OJ L 184, 12/07/1997, p. 33
[13] OJ L 70, 16.3.2005, p. 1

Directive 2002/32/EC	
ML for chlordane (sum of *cis*- and *trans*-isomers and of oxychlordane and expressed as chlordane), relative to a feedingstuff with a moisture content of 12 %	
Product	**mg/kg**
Fats	0.05
Other feedingstuffs	0.02

EU-Pesticide residue legislation	
MRL for chlordane (for products of plant origin: sum of *cis*- and *trans*-chlordane and for products of animal origin: sum of *cis*- and *trans*-isomers and of oxychlordane and expressed as chlordane) applicable to the product as marketed	
Product	**mg/kg**
Fruit and vegetables	0.01
Oilseeds	0.02
cereals	0.02
Meat (fat)	0.05
Milk	0.002
Eggs	0.005

The maximum levels for chlordane in Directive 2002/32/EC are as regards the major feed materials (cereals, oilseeds) comparable to those in the pesticide legislation.

TERMS OF REFERENCE AS PROVIDED BY THE REQUESTOR

In accordance with Article 29 (1) a of Regulation (EC) No 178/2002 the European Commission asks the European Food Safety Authority to provide a scientific opinion on the presence of chlordane in animal feed.

This scientific opinion should comprise the

- determination of the toxic exposure levels (daily exposure) of chlordane for the different animal species of relevance (difference in sensitivity between animal species) above which
  - signs of toxicity can be observed (animal health / impact on animal health),
  - the level of transfer/carry-over of chlordane from the feed to the products of
- animal origin results in unacceptable levels of chlordane or of its metabolites in the products of animal origin in view of providing a high level of public health protection.
- identification of feed materials which could be considered as sources of contamination by chlordane and the characterisation, insofar as possible, of the distribution of levels of contamination
- assessment of the contribution of the different identified feed materials as sources of contamination by chlordane
  - to the overall exposure of the different relevant animal species to chlordane,
  - to the impact on animal health,

- to the contamination of food of animal origin (the impact on public health), taking into account dietary variations and carry-over rates
- identification of eventual gaps in the available data which need to be filled in order to complete the evaluation.

ACKNOWLEDGEMENT

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ASSESSMENT

1. Introduction

Chlordane is a persistent chlorinated non-systemic (not taken up in the plant), contact and ingested insecticide, which was extensively used from 1947 onwards. Chlordane was mainly used as an agricultural insecticide but also for non-agricultural purposes mainly in the USA. From 1981, it was banned in the European Union and is now banned in most countries. Chlordane is not a single compound but a complex mixture of various constituents which might be transformed to different metabolites and breakdown-products. The constituents and transformation products are often termed "related compounds".

In the environment, chlordane and related compounds show high persistency and can undergo long-range atmospheric transport. Chlordane is included in the Stockholm convention on persistent organic pollutants (POPs)[14] and the United Nations Economic Commission for Europe (UNECE) Convention on long-range transboundary air pollution protocol on POPs (CLRTAP-POP)[15].

When the term chlordane is used without further explanation in this opinion, it refers to technical chlordane without knowledge of the exact composition of the mixture.

[14] http://www.pops.int/documents/convtext/convtext_en.pdf
[15] http://www.unece.org/env/lrtap/full%20text/1998.POPs.e.pdf

1.1. Synthesis and chemistry

Technical chlordane was first commercially produced in the USA in 1947 while it was first described as an insecticide in 1945 by Kearns (1945). This in turn started the development of other cyclodienes as pesticides (Kirk and Othmer, 1981).

Technical chlordane consists of at least 147 compounds, 120 of which have been identified (Dearth and Hites, 1991a). The composition varies with the manufacturing process and during the period 1950 - 1970 the formulation consisted of 43 – 75 % *cis*- and *trans*-chlordane and lesser amounts of heptachlor ($C_{10}H_5Cl_7$), nonachlor ($C_{10}H_5Cl_9$), and chlordenes ($C_{10}H_6Cl_6$). From the 1970s a more refined formulation (HCS 3260) containing more than 95 % chlordane was also produced with a ratio of *cis*- to *trans*-chlordane of about 3:1 (de Boer, 1999; Nomeir and Hajjar, 1987).

Technical chlordane is produced by Diels-Alder condensation of cyclopentadiene and hexachlorocyclopentadiene to form chlordene whereupon chlordene is further chlorinated at high temperature and pressure to chlordane (Dearth and Hites, 1991a). Figure 1 shows the reaction for the two predominant constituents of technical chlordane, *cis*-chlordane (1-*exo*,2-*exo*, 4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane; $C_{10}H_6Cl_8$) and *trans*-chlordane (1-exo,2-endo,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane; C10H6Cl8). *Trans*-chlordane has a greater insecticidal effect than the *cis*-isomer (Kirk and Othmer, 1981).

Figure 2 shows the IUPAC numbering system for the general chlordane structure

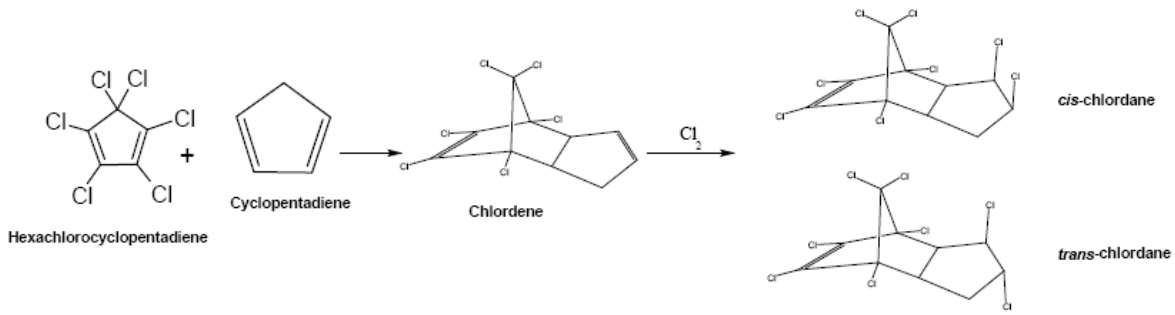


Figure 1. Synthesis of *cis*- and *trans*-chlordane with chlordene as an intermediate.

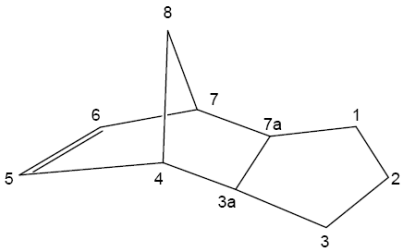


Figure 2. IUPAC-numbering system for chlordane-structures (3a,4,7,7a-tetrahydro-4,7-methanoindane).

In the literature, *cis*-chlordane is also known as α-chlordane while *trans*-chlordane is commonly known as γ-chlordane or even β-chlordane. This is particularly confusing since γ-chlordane is also the common name of another octachloroisomer (2,2,4,5,7,7,8,8-octachlordane) (ATSDR, 1994) as well as being the name of one of the technical chlordane products. In this opinion the terms *cis*- and *trans*-chlordane are used.

Technical chlordane is a colourless to amber to yellowish-brown viscous liquid with an aromatic, slight pungent odour similar to chlorine. The liquid is miscible with cyclohexanone, kerosene, petroleum solvents, 2-propanol, trichloroethylene, aliphatic and aromatic solvents. Solubility in water is 56 µg/L at 25°C. The melting point is <25°C, Henry's law constant 4.9 Pa m^3/mol at 25°C, log Koc = 4.58-5.57, log Kow = 6.00, and vapour pressure 1.3×10^{-3} Pa at 30°C (Montgomery, 1997).

According to Brooks (1974, as cited in Montgomery, 1997) the less refined technical chlordane has the approximate composition: *trans*-chlordane (24 %), four chlordene isomers (21.5 %), *cis*-chlordane (19 %), heptachlor (10 %), nonachlor (7 %), pentachlorocyclopentadiene (C_5HCl_5) (2 %), hexachlorocyclopentadiene (C_5Cl_6) (>1 %), octachlorocyclopentaene (C_5Cl_8) (1 %), $C_{10}H_{7\text{-}8}Cl_{6\text{-}7}$ (8.5 %) and other unidentified compounds (6 %). The compounds identified by Miyazaki *et al.* (1985) are assigned with "MC" (Miyazaki compound) as prefix combined with a number referring to the elution order, while those listed as unknown by Dearth and Hites (1991a) are designated by an "U" in front of a number, the first digit of which informs on the number of chlorine atoms and the second one represents the elution order. MC5 has been found to be 6.1 % of technical chlordane while U82 ($C_{10}H_6Cl_8$) and MC7 ($C_{10}H_6Cl_8$) were each found to represent 2.2 % of the mixture (Hoekstra *et al*., 2003). Buser and Müller (1993) analysed technical chlordane and found even lower amounts of chlordane in the mixture, the composition being 14.5 % *trans*-chlordane, 13 % *cis*-chlordane, 3.6 % MC5 ($C_{10}H_6Cl_8$), 10 % heptachlor, 6 % *trans*-nonachlor, 1.6 % *cis*-nonachlor and 0.5 % MC6 ($C_{10}H_4Cl_9$). Minor constituents of technical chlordane are compounds having 10 - 12 chlorines produced by the condensation of three cyclopentadiene molecules representing approximately 0.01 – 0.03 % of the technical mixture, but highly accumulating in the environment (Dearth and Hites, 1990). Miyazaki *et al.* (1985) found MC5 to be 2.3 % of the technical mixture, MC6 to be 0.8 % and MC7 to be 1.9 %. Additionally, unreacted chlordene is a minor component of technical chlordane, but caged hexachloro compounds resulting from Wagner-Meerwein rearrangement of chlordene exist, often named α-, β-, and γ-chlordene, compounds that have been detected in environmental samples (Dearth and Hites, 1991a; Simpson *et al.,* 1996). Hence these data clearly show that the composition of technical chlordane may vary considerably.

The structures of several constituents of technical chlordane, which have been found in environmental samples, are shown in Figure 3. Oxychlordane ($C_{10}H_4Cl_8O$) is the main metabolite of *cis*- and *trans*-chlordane as well as other octachlorinated and nonachlorinated chlordane compounds (Nomeir and Hajjar, 1987). Oxychlordane is very persistent and is depicted in Figure 4.

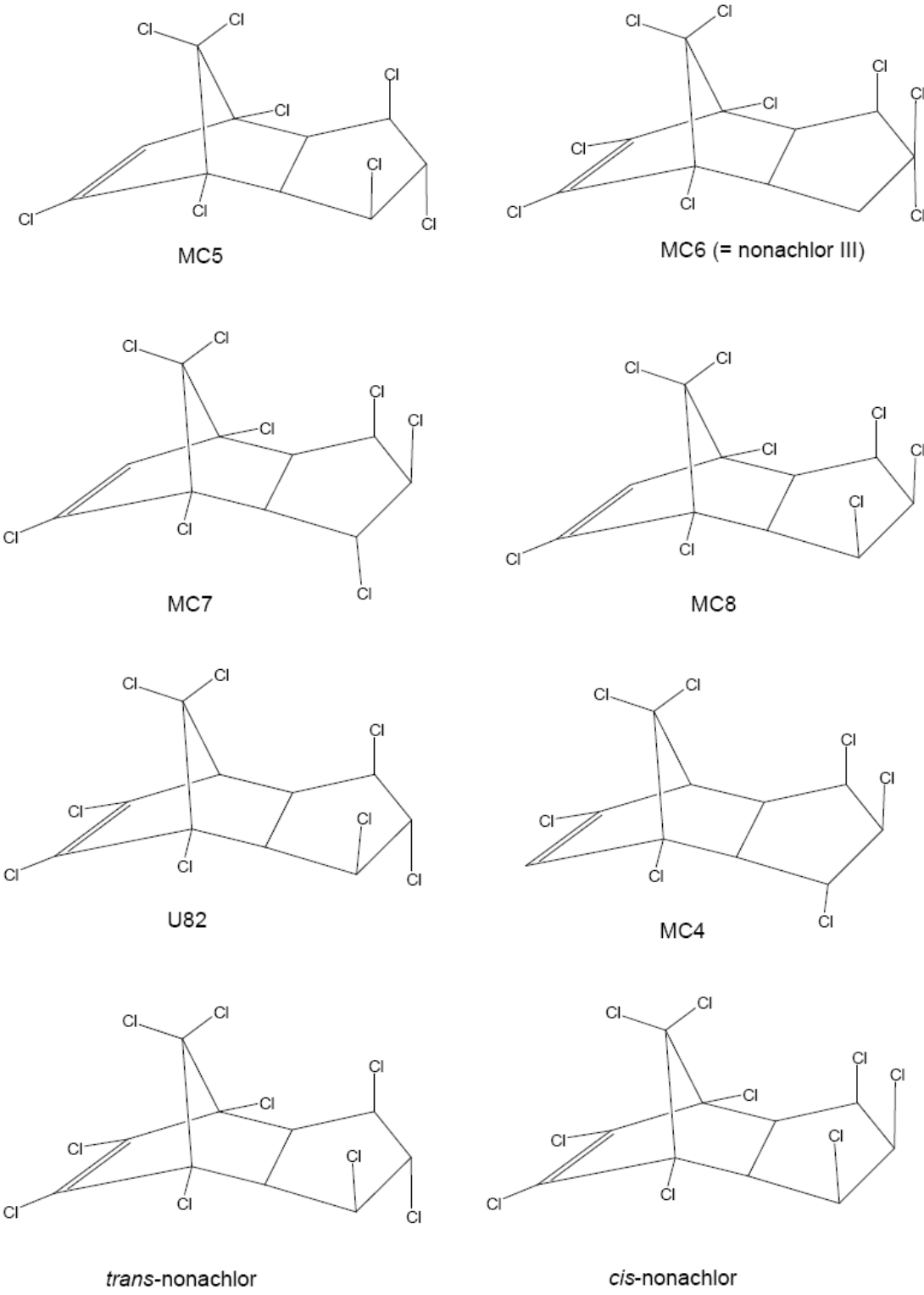


Figure 3. Structures of commonly studied chlordane components. The designation "MC" refers to Miyazaki compound since Miyazaki was the first to identify these (Miyazaki *et al*., 1985) and the number refers to elution order. The designation "U" in front of a number refers to "unknown" and the first digit informs on the number of chlorine atoms and the second one represents the elution order (Dearth and Hites, 1991a).

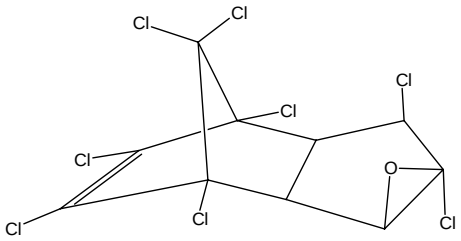


Figure 4. Structure of oxychlordane (2-*exo*, 3-*endo*, 4,5,6,7,8,8-octachloro-2,3-*exo*-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindane) according to Buser and Müller (1992).

Furthermore, *trans*- and especially *cis*-chlordane may undergo photolysis, particularly in the presence of photocatalysts, to form half-caged structures (Buser and Müller, 1993). Photo-*cis*-chlordane has been found to biomagnify in marine organisms (Strandberg *et al.,* 1998). Photo-*cis*-chlordane may exist in two structures as shown in Figure 5.

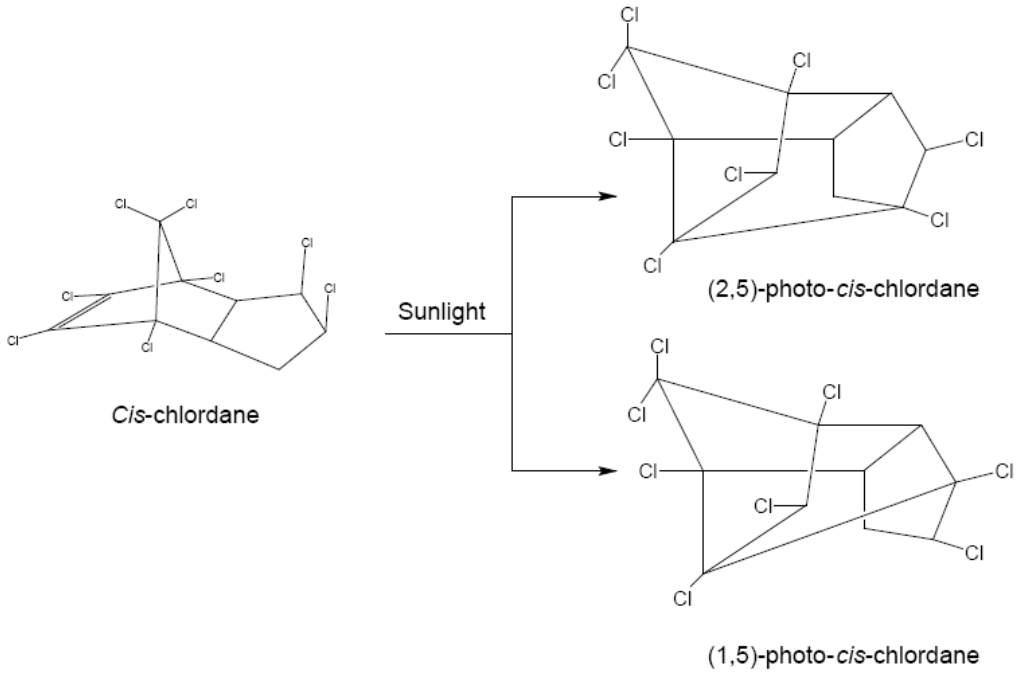
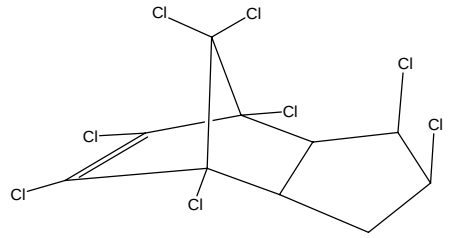
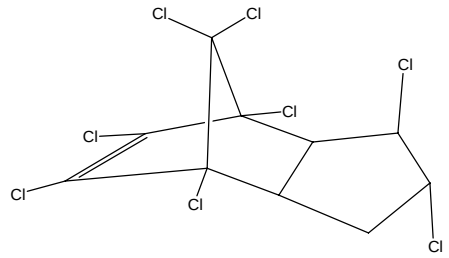
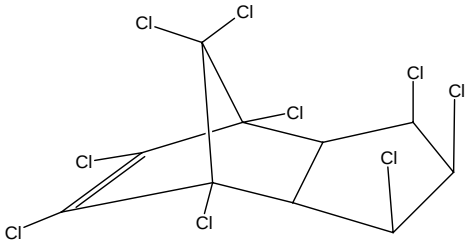
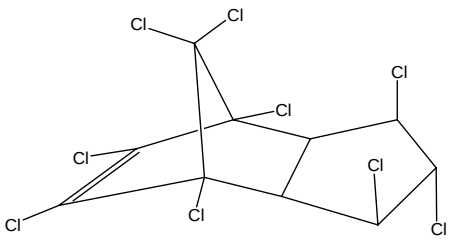


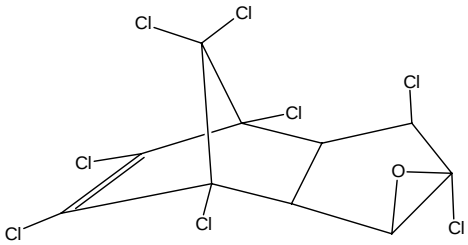
Figure 5. Suggested half-cage structures of photo-*cis*-chlordanes through C2-C5 and C1-C5 bond formation (Buser and Müller, 1993).

Except for *cis*- and *trans*-nonachlor, the chlordanes shown in Figure 1 and 3 as well as oxychlordane, and photo-*cis*-chlordanes, are chiral compounds, existing in two enantiomeric forms (Vetter and Schurig, 1997).

Physical and chemical properties of *cis*- and *trans*-chlordane, oxychlordane, and *cis*- and *trans*-nonachlor are summarised in Table 1. Except for heptachlor and heptachlorepoxide, for which data were presented in the opinion on heptachlor (EFSA, 2007), data on other chlordane compounds are scarce.

Table 1. Physical and chemical properties of some chlordanes

Structure and name	Properties
***Cis*-chlordane** 	Synonyms: 1-*exo*,2-*exo*, 4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane; α-chlordane. CAS Registry: 5103-71-9 Molecular mass: 409.8 Melting point (°C): 107.0 - 108.8[a] Solubility in water: 51 µg/L (20 - 25°C)[a] Vapour pressure, Pa (25°C): $4.8 \cdot 10^{-3}$ [a] Log Kow: 5.93[a], 6.10[b] Log Koc: 4.55 - 4.94[a] Henry's law constant, Pa·m^3/mol: 89[a] (23°C), 5.5[c] (25°C), 4.3[d], 5.7[e], 27[f]
***Trans*-chlordane** 	Synonyms: 1-*exo*,2-*endo*, 4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane; β-chlordane; γ-chlordane. CAS Registry: 5103-74-2 Molecular mass: 409.8 Melting point (°C): 103.0-105.0[a] Vapour pressure, Pa (25°C): $3.7 \cdot 10^{-3}$ [a] Log Kow: 8.69, 9.65(calculated)[a], 6.22[b] Log Koc: 4.67-5.04[a] Henry's law constant, Pa·m^3/mol: 136[a] (23°C), 15.9[c] (25°C), 6.8[e], 29.0[f]
***Cis*-nonachlor** 	Synonym: 1-*exo*,2-*exo*, 3-*exo*, 4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane. CAS Registry: 3734-49-4 Molecular mass: 444.2 Solubility in water: 8.2 µg/L[g] (25°C) (both *cis* and *trans*) Log Kow: 6.08[b], 5.7[i] Henry's law constant, Pa·m^3/mol: 0.60[d] (25°C)
***Trans*-nonachlor** 	Synonym: 1-*exo*,2-*endo*, 3-*exo*, 4,5,6,7,8,8-nonachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane. CAS Registry: 3734-49-4 Molecular mass: 444.2 Solubility in water: 8.2 µg/L[g] (25°C) (both *cis* and *trans*) Log Kow: 6.35[b], 32.0[f], 2.5[h], 5.7[i] Henry's law constant, Pa·m^3/mol:10.7[c] (25°C)

Structure and name	Properties
Oxychlordane 	Synonym: 1-*exo*,2-*endo*, 4,5,6,7,8,8-octaachloro-2,3-*exo*-epoxy-3a,4,7,7a-tetrahydro-4,7-methanoindane. CAS Registry: 27304-13-8 Molecular mass: 423.7 Vapour pressure, Pa (25°C): $9.2 \cdot 10^{-4}$ [j] Log Koc: 3.9[j] Henry's law constant, Pa·m^3/mol: $8.7 \cdot 10^{-3}$ [j]

a) Mongomery, 1997; b) Simpson, 1995; c) Cetin *et al.*, 2006; d) Altsuch *et al.*, 1999; e) Shen and Wania, 2005: f) Jantunen and Bidleman, 2006; g) HSDB, 2005; h) Meylan and Howard, 1991; i) Lebeuf *et al*. 2007; j) HSDB, 2003.

1.2. Production, use and environmental fate

1.2.1 Production and use

Chlordane was first commercially produced in the US in 1947. In 1974 the production in the US amounted to 9,500 tonnes per year. The cumulative global production has been estimated to be 70,000 tonnes (Dearth and Hites, 1991a; Christen, 1999). According to the WHO (1984) chlordane has not been produced in Europe or Japan. AMAP (2004) reported the existence of production facilities in Singapore and China, and according to IARC (2001) chlordane was manufactured in India and Argentina. The estimated recent annual production in China, by nine manufacturers in eastern China (Shanghai area), is 500 - 800 tons (Jaward *et al*., 2005).

In addition to chlordane, common and trade names have included 1,2,4,5,6,7,8,8-octachloro-3a,4,7,7a-tetrahydro-4,7-methanoindan, Belt, Chlor Kil, Chlordan, Clordano, Chlortox, Corodane, γ-Chlordan, Gold Crest C-50, Gold Crest C-100, Kilex, Kypchlor, M-410, Niran, Octachlor, Penticklor, Prentox, Synchlor, Termi-Ded, Termex, Topiclor 20, Toxichlor, Velsicol 168, and Velsicol 1068.

Chlordane was initially mainly used as an agricultural insecticide, but in the US up to 50 % could have been used for non-agricultural purposes (as a household pest control, lawn protection, etc.). Outside the US the non-agricultural use has been estimated to about 10 %. In Japan for example, 240 tons of chlordane were used as an agricultural insecticide up to 1968 and 17000 tons were used as a termiticide for housing and wood preservation by 1986 (Jawata *et al*., 2005). In Europe it was mainly used for the protection of crops of potatoes, vegetables, small grains and sugar beets (FAO/WHO, 1971), although no information on the amounts used has been identified. Chlordane has also been used as an ingredient in veterinary preparations for the protection of livestock from different pests.

Similar to heptachlor, chlordane is used as a non-systemic (not taken up by the plant), contact and ingested insecticide. It has been shown to non-competitively block GABA receptors in

cockroach, *Periplaneta americana,* and other insects. It is non-phytotoxic at insecticidal concentrations (Worthing and Walker, 1987).

The use of chlordane as a pesticide has been banned in the EU since 1981[16]. In the US most uses were phased out in 1978 and the remaining uses were phased out by 1988, but the production for export was continued until 1997. In Canada, registration of chlordane was discontinued in 1990. The Stockholm Convention prescribes each party to prohibit and/or take the legal and administrative measures necessary to eliminate production and use as well as import and export of chlordane.

According to UNEP Chemicals (UNEP, 2002) summarising legal status for a number of POPs in about 110 countries, chlordane was still allowed in four countries and in a further 12 countries use was still permitted but with restrictions. Common restrictions were that chlordane could be used for pre-construction protection of building and installation material or that only a defined amount still in stockpiles could be utilized. Another reason for not reporting a ban is incomplete legislation (e.g. on use) as a few countries without a ban on use report both no national production and a ban on import. However, the fact that a country reported no specific regulation for a pesticide does not necessarily mean that this pesticide was used in that country. In fact, a number of countries stating lack of specific legislation on chlordane also report that they have not found the compound within the country.

The general picture is that chlordane has very limited use, if any, today. An exception is China from where it has recently been reported that chlordane is extensively used against termites with an estimated amount of over 200 tons/year (Xu *et al.,* 2004). Furthermore, particularly outside Europe it appears that there are still considerable amounts in wooden building materials as well as in stockpiles that all eventually could contaminate the environment.

1.2.2. Environmental fate

In air, chlordane exists predominantly in the vapour phase. However, for atmospheric deposition, particle-bound chlordane appears to be of major importance (Atlas and Giam, 1988). In the vapour-phase, chlordane degrades by photolysis and hydroxyl radical reactions. On the other hand, photo-reactions also generate stable half-caged photo-isomers (see also Chapter 1.1.). Chlordane may be transported over long distances and could be deposited by wet as well as dry deposition. As a result of its long range transport properties, chlordane is found world-wide and is prevalent in Arctic food webs (AMAP, 2002).

In an attempt to describe the distribution of organochlorine compounds in the atmosphere and hydrosphere, air and surface seawater samples were collected 1989-1990 from several oceans

[16] Council Directive 79/117/EEC of 21 December 1978 which prohibited the placing on the market and use of plant protection products containing certain substances

and analysed inter alia for various chlordane compounds. Concentrations of chlordane, expressed as the sum of *cis*-chlordane, *trans*-chlordane and *trans*-nonachlor were higher in the atmosphere of the Northern Hemisphere than in the Southern Hemisphere. It was found that the ratio of specific chlordane compounds can be used as a complement tool to understand the emission source areas of global contamination. As technical chlordane generally has a ratio for *trans*-nonachlor/*trans*-chlordane of 0.15 – 0.45, continuous usage of chlordane would result in a similar ratio in the environment. This was substantiated by results of air samples from the Northern Hemisphere. Considerably higher ratios were found in air samples far away from possible sources, indicating the preferential depletion of trans-chlordane (Iwata *et al.*, 1993). Recent surveys of air concentrations at 40 sites globally (7 continents) confirm these observations. Higher levels in Arctic air than in Antarctic air indicate recent uses (*i.e.* ratio of *trans*-chlordane to *cis*-chlordane higher than 1.54 as in the technical mixture) in areas with highest concentrations (sum of *cis*- and *trans*-chlordane and *trans*-nonachlor), *e.g.* Phillipines, Darwin in Australia, and Chengdu, China (Pozo *et al.,* 2006). Additionally, in Asia (77 sites in China, Japan, S-Korea, and Singapore), the highest air levels of chlordane (sum of *cis*- and *trans*-chlordane) in 2004 were generally found in Japan and some Chinese locations (Jawarda *et al*., 2005). However, Kallenborn *et al.* (1998) found comparable levels of chlordane (*cis*- and *trans*-chlordane, and *cis*- and *trans*-nonachlor) at Signy Island, Antarctica, as in the Arctic.

The air levels of chlordane in the Arctic (*cis*- and *trans*-chlordane, and *trans*-nonachlor) decreased between 1984 and 1998 with apparent half-times of 4.9 - 9.7 years. Also the ratio of *trans*-chlordane to *cis*-chlordane decreased reflecting aging sources (Bidleman *et al*., 2002).

Chlordane is highly persistent in soil and generally, half-lives around 4 years have been reported. Several studies have found chlordane residues of more than 10 % of the initially applied amount 10 years or more after application (ATSDR, 1994). Evaporation is the major route of removal from soils. As chlordane is adsorbed to soil particles in the top soil layers it volatilises slowly from soil into the atmosphere.

Also in water, chlordane is relatively stable. It can escape from the water compartment by adsorbing to sediments or by volatilization from water surfaces. The volatilization half-life for chlordane in lakes and ponds is estimated to be less than 10 days. The presence of chlordane in drinking water is almost always due to accidents (ATSDR, 1994).

Bioaccumulation and bioconcentration

Biomagnification of chlordane is a complex issue as the compositional pattern (including metabolites) varies with trophic level and food chain (Kawano *et al.*, 1988). This is due to differences in uptake and metabolism of the different constituents of the technical mixture. Accumulation and transformations of chlordanes have been shown to be enantiomer-selective (Wiberg *et al.,* 1998; Klobes *et al.,* 1998; Hoekstraa *et al.,* 2003a; Hoekstra *et al.*, 2003b;

Wiberg *et al.*, 2000; Warner and Wong, 2006; Buser and Müller, 1994). For example, the enantiomeric ratio in cod has been shown to depend even on gender (Karlsson *et al.,* 2000). As a general rule for environmental samples, the deviation of the enantiomeric fraction from 0.5 (racemate) show similar patterns, i.e. air < water < soil < biota (Hegeman and Laane, 2002). In biota, the order is: lower trophic level < higher trophic level and liver or kidney tissue < brain tissue. Recently, information on enantiomeric fractions in reference materials, such as fish homogenates, has been made available (Wong *et al.,* 2002).

Generally, the composition of chlordane compounds changes in comparison with the technical mixtures when moving up the food chain. *Trans*-nonachlor and in particular oxychlordane concentrations increase at higher trophic levels (Dearth and Hites, 1991b). For example, marine invertebrates and most fishes show similar chlordane composition to that of technical chlordane, whereas marine mammals, humans and birds show altered chlordane composition in their tissues (Muir *et al,.* 1988; Norstrom *et al.,* 1988; Dearth and Hites, 1991b). Additionally, in mammals, such as seals, polar bears and humans, representing the highest trophic level, U82 and MC5 (Figure 3) are the most abundant of the octachloro compounds present in technical mixtures (Dearth and Hites, 1991b; Karlsson *et al.*, 1999; Hoekstra *et al.*, 2003a). These two compounds show high accumulation factors with an accumulation factor for U82 42 times higher than that of *cis*-chlordane (Dearth and Hites, 1991b). It has been suggested that the *trans*-configuration, i.e. endo-chlorine at position C2 and exo-chlorines at C1 and C3 may be the reason for a high accumulation (Karlsson *et al.,* 1999). This configuration is the same as for *trans*-nonachlor and MC5, which both accumulate more in human tissues than *cis*-nonachlor, and MC7, which have chlorine in exo-configuration at C2 (Dearth and Hites, 1991b; Zhu *et al.,* 1995). This is further evidenced by the fact that *trans*-nonachlor and U82 both have similar half-lives, 15 and 16 days, respectively, in rats, only exceeded by U81 (octachlordane, structure not known) and MC6 (nonachlordane) (>30 days) (Dearth and Hites, 1991c).

In fish, chlordane shows a high potential for bioaccumulation with bioconcentration factors (BCFs) between 3000 and 18,500 (Zaroogian *et al.,* 1985; Oliver and Niimi, 1985). Biomagnification of chlordane expressed as the sum of 12 different chlordane compounds has been studied in a marine food chain (cod - ringed seal - polar bear) resulting in a fish to polar bear biomagnification factor of 44.2 (Muir *et al.*, 1988). In a marine food web of the Barents Sea (zooplankton, ice fauna, fish (poikilotherms) as well as seabirds and seals (homeotherms)), the concentrations of chlordane (*cis*-chlordane, *trans*-nonachlor, and oxychlordane) in the homeotherms were orders of magnitude higher than in the poikilotherms (Hop *et al*., 2002). Furthermore, the rate of increase with trophic level, *i.e.* the food web biomagnification factor, was significantly higher among homeotherms than poikilotherm, and especially high for *cis*-chlordane due to higher energy requirement and greater biotransformation capabilities of the former (Hop *et al*., 2002). In polar bears, adult males eliminate chlordane more efficiently than females in contrast to what is found for most other organochlorine compounds (Bernhoft *et al.*, 1997).

Levels in the environment

Ambient air concentrations of chlordane mostly from the USA, are generally reported to be in the picogram per m^3 range (Bidleman *et al.,* 1992; Knap and Binkley, 1991; Patton *et al.,* 1991). Following use, elevated levels in air have been reported from Africa and the USA (Jantunen *et al.,* 2000; Karlsson *et al.,* 2000). The finding of chlordane with unaltered technical chlordane composition in air of East Asia in 2004 (Primbs *et al*., 2007) might reflect recent and extensive use in China (see above). High values in the microgram per m^3 range have been reported from many areas in the USA, especially in indoor air in buildings treated with chlordane or urban air in areas where chlordane has been poured or injected into soil around the foundation of houses. Such levels were also found in areas where chlordane was previously used as a pesticide and where it is still being released from the soil (Andersson and Hites, 1989; US-EPA, 1990).

Residues of chlordane compounds (sum of *cis*- and *trans*-chlordane, *trans*-nonachlor and oxychlordane) were determined in a number of pooled samples from several fish species of the Northeast Atlantic by Karl and co-workers (1998). The sum (mean values) varied from <0.01 to 8.4 µg/kg fresh weight in the edible part of the fish. The higher levels were found in species with high fat content: eel (4.5 µg/kg fresh weight), Greenland halibut (8.4 µg/kg fresh weight), salmon (5.1 µg/kg fresh weight), herring (2.7 µg/kg fresh weight) and redfish (2.4 µg/kg fresh weight). Angler and Bonito did not contain detectable levels with LODs of 0.01 µg/kg wet weight for each of the four compounds for these species. *Trans*-nonachlor and *cis*-chlordane were generally at similar levels in most of the fish species and about five times higher than both oxychlordane and *trans*-chlordane.

Falandysz and coworkers (2000) analysed the concentration and composition of chlordane compounds in several fish species caught in the Gulf of Gdańsk. The analytical method comprised *cis*-chlordane, *trans*-chlordane, *cis*-nonachlor, *trans*-nonachlor, oxychlordane, heptachlor, heptachlor epoxide, MC4, MC5, MC6, MC7, U82 and U83. All chlordane compounds were found in the fish samples with levels (sum of the above compounds) between 0.40 and 12 µg/kg fresh weight. In almost all samples *trans*-nonachlor gave the highest concentration followed by *cis*-chlordane and oxychlordane. MC5 and MC6 amounted on average 5 – 8 and 2 – 5 %, respectively to the sum of all chlordane compounds Lower contributions to the sum of chlordane compounds were found for MC4, MC6 and U82 with values below 2 %.

Cis- and *trans*-chlordane and *trans*-nonachlor have also been found in toothed whales such as the Northwest Atlantic Right Whales (*Eubalaena glacialis*), with mean concentrations in the range of 170 to 3000, 90 to 1800 and 90 to 1900 ng/g lipid weight of *cis*- and *trans*-chlordane and *trans*-nonachlor, respectively. These levels were similar to those of PCB 153 in the same animals. In Northwest Atlantic pilot whales (*Globicephala melas*), mean values of *cis*- and *trans*-chlordane and *trans*-nonachlor have been reported to be in the range of 300, 50 and

1850 ng/g lipid weight respectively. Levels of PCB 153 were reported in the range of 1500 ng/g lipid weight (Weisbrod *et al.*, 2000a,b).

Generally, the levels of chlordane are slowly decreasing in the environment globally. However, exceptions are found, *e.g.* increase in MC6 and *trans*-nonachlor in the marine pelagic food web in the Antarctic between 1987 and 1996 (Goerke *et al*., 2004).

1.3. Toxicology in laboratory animals and hazard assessment for humans

Chlordane has been evaluated several times by various international bodies (WHO-IPCS, 1984; FAO/WHO, 1987, 1994; IARC, 2001; WHO, 2004), and by the Agency for Toxic Substances and Disease Registry (ATSDR, 1994). Most toxicological studies, particularly the early ones, have been carried out with technical chlordane often with different and poorly defined composition (WHO, 2004).

Chlordane has moderate acute toxicity with oral LD_{50} values for mice and rats of 335 - 430 mg/kg b.w., and higher values (1720 mg/kg b.w.) in the hamster, *Cis*-chlordane is more toxic than *trans*-chlordane. Oxychlordane has high acute toxicity with a LD_{50} in the rat of 20 mg/kg b.w. Other constituents of the technical mixture and metabolites tested (chlordene, 3-chlorochlordene, 1-hydroxychlordene, chlordene epoxide, 1-hydroxy,2,3 epoxychlordene, 2-chlorochlordene) have lower acute toxicity than chlordane (WHO, 1984, 2004).

The target organ for the acute toxicity of chlordane is the central nervous system, and symptoms such as disorientation, ataxia, tremors, convulsions and respiratory failure and cyanosis have been reported (WHO, 1984, 2004). In humans neurological symptoms, including headache, dizziness, vision problems, incoordination, irritability, excitability, weakness, muscle twitching and convulsions have been described in case reports on accidental acute exposures (WHO, 1984, 2004).

Upon low-level longer-term exposure, the liver is the target for toxicity of chlordane with induction of hepatic microsomal enzyme activity as one of the most sensitive biochemical parameters. At higher levels, liver hypertrophy with histopathological and functional changes may occur (WHO, 1984, 2004).

In a 28 day study in rats, *cis*-nonachlor, *trans*-nonachlor and technical chlordane were compared for toxicity (Bondy *et al.*, 2000). For all the chemicals tested, oxychlordane was the major metabolite accumulating in the adipose tissue and most pronounced in females. The liver was the target organ in both sexes and hepatic changes were most pronounced in the rats exposed to *trans*-nonachlor. In males, kidney weights were increased and inhibition of the organic ion transporter were observed. Approximate toxicity ranking was *trans*-nonachlor > technical chlordane > *cis*-nonachlor. In a 90 day study in rats (Bondy *et al.,* 2004), *trans*-nonachlor at doses of 5 to 50 mg/kg feed caused phenobarbital like enzyme induction in the

liver at the lowest dose and histopathological changes at higher doses in the liver, thyroid gland and adrenals. In female rats there was a shift in the oestrus cycle at the highest dose.

Oxychlordane was gavaged to female rats at doses of 0.01, 0.1, 1, 2.5 or 10 mg/kg b.w. in a 28 day study (Bondy *et al.,* 2003). Weight loss, reduced feed consumption and thymic atrophy were major effects at the highest dose, whereas hepatic changes indicative of microsomal enzyme induction were observed at 2.5 mg/kg b.w. and above. The dose response curve was steep with 10 mg/kg b.w. causing acute toxicity with 100 % morbidity after one dose and 15 % weight loss already after 3 days when dosing was stopped. In contrast, 1 mg/kg b.w. had no measurable effect. The doses of 1 and 2.5 mg/kg b.w. resulted in adipose tissue levels of 134 and 269 mg oxychlordane/kg. The findings are compatible with those of Ivie (1973) who reported that oxychlordane was considerably more toxic in mammals than the parent *cis*- and *trans*-chlordane. He did not give details regarding species, route of exposure or dose. The most sensitive indicator of morbidity was reduced feed consumption accompanied by weight loss.

1.3.1. Long-term studies of toxicity and carcinogenicity

Long-term studies with oral chlordane exposure lasting for 2 years or more have been carried out in dogs (see chapter 5.8) mice and rats (WHO, 1984; FAO/WHO, 1987, 2004). In all studies the liver was the target organ showing hypertrophy with histopathological changes depending on dose ranging from liver cell hyperplasia to cell necrosis, fatty infiltration and, particularly in mice, nodule formation. The doses of chlordane associated with no adverse effects (NOAEL) were 3 mg/kg diet in beagle dogs, and 1 mg/kg feed in rats and mice, equivalent to 0.075, 0.05 and 0.12 mg/kg b.w. per day, for dogs, rats and mice respectively.

Technical-grade chlordane has been tested for carcinogenicity by oral administration in several strains of mice and rats. Chlordane at doses from 5 to 80 mg/kg diet, (equivalent to 0.5 to 8 mg/kg b.w. per day) increased incidences of hepatocellular neoplasms (including carcinomas) in both male and females of three strains of mice, one of which has a very low frequency of spontaneous liver lesions (Epstein, 1976; NCI, 1977; Becker and Sell, 1979). In three strains of rats, chlordane did not exhibit carcinogenic effects (Ingle, 1952; NCI, 1977; Research Institute for Animal Science in Biochemistry and Toxicology, 1983). Technical-grade chlordane marginally increased the incidence of hepatocellular adenomas in F-344 SPF male rats (Ihui *et al.,* 1983, cited in FAO/WHO 1987). Increased incidences of thyroid follicular-cell adenomas and carcinomas were seen in one study with chlordane in rats.

In initiation–promotion studies in mice, administration of chlordane after N-nitrosodiethylamine resulted in increased incidences of hepatocellular tumours (FAO/WHO, 1987; WHO, 1984).

1.3.2. Genotoxicity

Chlordane was mutagenic in yeast (*Saccharomyces cerevisiae*) and maize, but not in bacteria. Chlordane was mutagenic to Chinese hamster V79 cells and induced sister chromatid exchange in intestinal cells of fish treated *in vivo*. Chlordane was negative for unscheduled DNA synthesis in primary cultures of rat, mouse and hamster hepatocytes and in mice for the dominant lethal assay. Chlordane was not mutagenic to cultures of human fibroblasts. Studies on DNA damage in transformed human cells yielded conflicting results (WHO, 1984; IARC, 2001; WHO, 2004).

No data were available on genetic and related effects of chlordane in humans.

Chlordane is not mutagenic *in vivo* and not or only weakly mutagenic in a few tests *in vitro*.

1.3.3. Other cellular and biochemical effects

Chlordane inhibits gap-junctional intercellular communication (Ruch *et al.,* 1990) with an IC_{50} in rat liver epithelial cells IAR6.1 of about 7 μM (Rivedal and Witz, 2005).

In cell lines transfected with reporter genes, chlordane bound to and also activated retinoid receptors RARβ and RARγ, but not RARα or the RXR pathway (Lemaire *et al.,* 2005). Chlordane also induced PXR dependent CYP3A4 gene expression in hepatic cells and caused ERα dependent down regulation of CYP1A1 in mammary cells (Coumoul *et al.,* 2002).

In cells *in vitro* both chlordane (composition not given) and *trans*-nonachlor have shown estrogenic effects. Both activated ERα and ERβ in cell lines transfected with reporter genes, *trans*-nonachlor being slightly more potent. The estrogenic potencies of these compounds were more than three orders of magnitude below that of 17β-estradiol (Lemaire *et al.*, 2006). *Trans*-nonachlor, but not *cis*-nonachlor, bound to the human progesterone receptor (Scippo *et al.,* 2004). In a yeast based assay system, chlordane (composition not given) had an antagonistic effect on the EERα-1 orphan receptor, which may result in suppression of aromatase (*CYP 19*) expression (Chen *et al.*, 2001). However, induction of aromatase by chlordane was observed in the human choriocarcinoma JEG-3 cell line (Laville *et al.*, 2006).

1.3.4. Epidemiological studies on cancer

Several cohort studies, with different inclusion criteria and lengths of follow-up, have been conducted to investigate the mortality of workers producing chlordane and also other chemicals in the USA. In chlordane exposed workers, no excess was seen in the rate of mortality from all cancers (IARC, 2001).

A number of case–control studies were conducted to investigate the risks for cancers of the lymphohaematopoietic system, breast and a few other sites in relation to exposure to

chlordane. These studies differed widely in size and populations and in methods, including exposure assessment, which in some studies included the measurement of chlordane in samples of fat tissue or blood. In most studies, exposure to other organochlorines or other types of pesticides was also assessed. Four case–control studies of non-Hodgkin lymphoma showed a consistent but modest increase in the risk associated with exposure to chlordane, although it was almost impossible to separate the effect of chlordane from those of other pesticides as well as other exposures. In case–control studies addressing leukaemia, soft-tissue sarcoma, multiple myeloma, breast-, endometrial- and pancreatic cancer, no associations with chlordane were found (IARC, 2001). Five cases of neuroblastoma associated with self-reported domestic pre- and postnatal exposure to chlordane have been reported (Infante *et al.,* 1978). In two cases exposure to other agents including x-rays were noted. Based on all information, IARC (2001) concluded that the evidence for carcinogenicity in humans was inadequate.

1.3.5. Reproductive/developmental toxicity

In male rats exposed to chlordane (19.5 mg/kg diet, equivalent to 1 mg/kg b.w. per day) for 90 days, biochemical changes (up-regulation of nuclear androgen receptor sites and reduction in RNA and DNA content) in ventral prostate were observed (Shain *et al.,* 1977). In multigenerational studies in rats and mice, reduced litter viability and delayed growth with a NOAEL of about 30 mg chlordane/kg diet was observed. Whereas effects in the first generation occurred at higher doses (100 mg/kg b.w. in mice and 320 mg/kg b.w. in rats) (WHO, 1984), in the third and fourth generation, significant effects appeared at lower doses of 50 mg/kg feed. In female mice exposed during pregnancy to 8 mg/kg b.w., the progeny had reduced cell-mediated immunity at adult age (WHO, 1984, 2004).

There were no indications of teratogenicity.

1.3.6. Immune effects

The immunotoxicity of *cis*- and *trans*-nonachlor and technical chlordane were investigated in adult male and female Sprague–Dawley rats following a 28-day oral (gavage) treatment. The doses used were 0.25, 2.5 or 25 mg/kg b.w. per day. The immunologic endpoints included: quantification of the total serum immunoglobulin levels and flow cytometric analysis of peripheral blood leukocytes and T-lymphocyte subsets, evaluation of mitogen responses and natural killer (NK) cell activity. Delayed-type hypersensitivity response to oxazolone, and resistance to *Listeria monocytogenes* were examined in female rats treated with *cis*- or *trans*-nonachlor. Exposure levels of 2.5 and 25 mg/kg b.w. of these compounds caused significant effects on a number of immunologic endpoints, i.a. immunoglobulin levels, increased number of lymphocytes, and reduced resistance to *Listeria monocytogenes*. The NOAELs for the

immune effects were 0.25 mg/kg b.w. per day and above those reported for liver effects following long-term exposure in rats, mice or dogs (see section 1.3.1). In comparison with technical chlordane, *cis*- and *trans*-nonachlor were more immunotoxic (Tryphonas *et al.*, 2002).

1.3.7. Evaluations

Chlordane was classified by IARC (2001) as possibly carcinogenic to humans (Group 2B).

JMPR re-evaluated its earlier assessments on chlordane in 1986 (FAO/WHO, 1987) and established an ADI of 0.5 µg/kg b.w. by applying an uncertainty factor of 100 to a NOAEL of 50 µg/kg b.w. per day for liver toxicity in a long-term study in rats. In 1994, JMPR converted the ADI into a provisional tolerable daily intake (PTDI) with the same value (FAO/WHO, 1995).

Chlordane is not mutagenic *in vivo* and not or only weakly mutagenic in a few tests *in vitro*. It is a promoter of liver tumours in vivo and exhibit biochemical properties shared by many promoters of liver tumours.

2. Methods of analysis

According to Article 11 of Regulation (EC) No 882/2004[17] of the European Parliament and of the Council on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules, analysis methods used in the context of official controls shall comply with relevant Community rules or, (a) if no such rules exist, with internationally recognised rules or protocols, for example those that the European Committee for Standardisation (CEN) has accepted or those agreed in national legislation; or, (b) in the absence of the above, with other methods fit for the intended purpose or developed in accordance with scientific protocols.

Contrary to a number of other undesirable substances, no fixed analytical methods are prescribed by the European Commission for the determination of chlordane compounds in animal feed. Multi-residue procedures for polychlorinated biphenyls (PCBs) and pesticides including *cis*- and *trans*-chlordane as well as the chlordane metabolite oxychlordane in animal feeding stuffs, using HRGC-ECD and HRGC-MS are currently elaborated by the Technical Committee CEN/TC 327 "Animal feeding stuffs – methods of sampling and analysis" of the European Committee for Standardization (CEN, 2005). The methods are applicable to animal feeding stuffs with a water content up to about 20 % and a fat content up to about 10 %. The limits of quantification (LOQ) for each of *cis*- and *trans*-chlordane and the metabolite

[17] OJ L 165, 30.4.2004, p. 1–141

oxychlordane in feed by applying HRGC-ECD and HRGC-MS are given as 2.0 and 0.5 µg/kg, respectively. These methods are also capable of analysing *cis*- and *trans*-nonachlor with little extra effort.

A number of other well-proven, validated multi-residue methods are available for the quantitative determination of chlordanes in various environmental matrices, including food, feed and other biological specimens (Muir and Sverko, 2006). Most frequently *cis*- and *trans*-chlordane, *trans*-nonachlor and the metabolite oxychlordane are analysed for, but sometimes also *cis*-nonachlor and occasionally more chlordane compounds are identified such as U82, MC2, MC5, MC7, MC8 and photo-*cis*-chlordane. In humans and seals U82 and MC5 are the most abundant octachloro chlordane components (Dearth and Hites, 1991b; Karlsson *et al.*, 1999; Hoekstra *et al.*, 2003a).

Depending on the type of feed material, both of plant or animal origin, extraction as well as the extent of necessary subsequent clean-up steps may differ considerably. While after grinding solid materials are commonly extracted with boiling organic solvents using conventional Twisselmann, Soxhlet, accelerated solvent extraction (ASE) or microwave assisted extraction (MAE) procedures or by supercritical fluid extraction (SFE), liquid samples are mostly extracted by liquid/liquid partitioning. Co-extracted fat and other compounds which potentially may disturb the determination of chlordanes can be removed by gel permeation chromatography (GPC) and by adsorption chromatography on various solid phase materials (SPE), such as Florisil or alumina.

Due to the high electro negativity caused by the seven or more chlorine atoms of chlordane and related compounds, high-resolution gas chromatography with electron capture detection (HRGC-ECD) is the analytical method most commonly used. An efficient separation of chlordane compounds from other interfering substances, such as other organochlorine pesticides and PCBs is especially important when using HRGC-ECD. The gas chromatographic separation on two capillary columns of different polarity in routine monitoring programmes is therefore mandatory. Potential co-elution problems can also be overcome by applying combined high resolution gas chromatography with mass spectrometry (HRGC-MS) either in the electron impact (EI) or negative chemical ionization (NCI) mode. In addition to increased selectivity, mass spectrometric methods in general offer the possibility of performing the analyses by isotope dilution using ^{13}C-labeled internal standards. Because these compounds can be added to the samples at the very beginning of the analytical procedure and behave as the native analytes, they allow a valuable control on the losses during the analytical procedure and thus significantly increase the accuracy of the results.

Inter-laboratory studies have been conducted for organochlorine pesticides in biological samples since 1969 without noticeable general improvement in the coefficient of variation between laboratories (de Boer and Law, 2003; Villeneuve *et al.*, 2004). There are several reasons for this lack of improvement during the last 30 years. One of the main reasons is probably a decrease in concentrations in the tested materials. For example in QUASIMEME

(Quality Assurance of Information for Marine Environmental Monitoring In Europe), the coefficient of variation of *trans*-nonachlor, the only chlordane compound reported in that programme, is 32 and 26 % at assigned levels of 35 and 361 μg/kg cod liver oil, respectively, while in mussels, plaice and mackerel at assigned values of 0.06, 0.22 and 1.55 μg/kg resulted in coefficients of variation of 85, 34 and 56 %, respectively (de Boer and Wells, 1997). These authors concluded that the currently available analytical methods for organochlorine pesticides do not allow the production of very accurate results when analyte concentrations are below 1 μg/kg. Recent reports on results obtained in inter-comparisons among laboratories show that analysis of chlordane (*cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor) is still difficult for many laboratories (Carvalho *et al.,* 1999; Villeneuve *et al.*, 2004). For example, only 13 out of 55 laboratories in a world-wide inter-laboratory study analysing fish homogenate for chlordane compounds provided acceptable results for *cis*-chlordane at median levels of 0.28 μg/kg dry weight while only 3 out of these 55 provided acceptable data for *cis*- and *trans*-nonachlor at median levels of 0.86 and 4.1 μg/kg dry weight, respectively (Villeneuve *et al.,* 2004). Similar outcome was obtained in an interlaboratory study for *cis*-chlordane in dried seaweed at median levels of 1.4 μg/kg dry weight where only 8 laboratories out of 80 produced reliable data (Carvalho *et al.,* 1999). In both these studies, only indicative values could be assigned for the chlordane compounds. Therefore, increased efforts for the improvement in analytical performance are highly recommended. However, current maximum levels in feed are well above the levels associated with high uncertainty. Additionally, matrices with reported low levels of chlordane compounds should be used with caution in for example exposure assessments.

In the present analytical scheme *cis*- and *trans*-chlordane as well as the chlordane metabolite oxychlordane are included. However, both *cis*- but especially *trans*-nonachlor occur at relatively high proportions particularly in feed samples of marine origin. Therefore, the Panel suggests that these should also be included in monitoring programmes especially for feedingstuffs of marine origin. Moreover, data indicate that U82 and MC5 should also be included in the analysis of chlordane compounds.

3. Statutory limits

The use of chlordane as a pesticide has been banned in the EU since 1981 by Council Directive 79/117/EEC[18] (see also Regulation 850/2004/EC[19]) which prohibited the placing on the market and use of plant protection products containing certain substances. Chlordane (sum of *cis*- and *trans*-isomers and of oxychlordane, expressed as chlordane) is listed in the Annex to Directive 2002/32/EC. The maximum levels which apply to the sum of *cis*- and *trans*-

[18] OJ L 33, 8.2.1979, p. 36
[19] OJ L 229, 29.6.2004, p. 5

chlordane and oxychlordane, expressed as chlordane each pertain to a feedingstuff with a moisture content of 12 %. See also specific background.

The Codex Alimentarius Commission adopted "extraneous maximum residue limits" (EMRL) for chlordane (defined as sum of *cis*- and *trans*-chlordane for plant commodities and sum of *cis*- and *trans*-chlordane and oxychlordane for animal commodities)[20]. An EMRL refers to a pesticide residue or a contaminant arising from environmental sources (including former agricultural uses) other than the use of a pesticide or contaminant substance directly or indirectly on the commodity. It is the maximum concentration of a pesticide residue or contaminant that is recommended by the Codex Alimentarius Commission to be legally permitted or recognised as acceptable in or on a food, agricultural commodity, or animal feed.

4. Occurrence in feed and animal exposure

Chlordane belongs to the group of undesirable substances, which are routinely analysed in the Member States within the framework of official feed controls. The aim of these monitoring programmes is to check compliance with legal limits laid down in the Annex to Directive 2002/32/EC. Unfortunately, a lot of information on the actual contamination of feeding stuffs regarding names of detected pesticides as well as their determined amount is not communicated because the Commission only requests the Member States to report their results in a condensed form as compliant or non compliant. Furthermore, it is often not specified in the condensed reports which compounds are covered by the analytical methods in the different Member States nor are the limits of detection reported. Finally, in many cases it is difficult to differentiate between numbers of individual analyses on the one hand and number of samples on the other hand. Consequently, for an evaluation of the occurrence of specific undesirable substances in feed as a prerequisite for a meaningful risk assessment, a number of subsequent queries in the Member States could be avoided if the occurrence data were to be reported in a more detailed form.

As an insecticide, chlordane is predominantly applied as a spray or powder. Therefore, vegetables and crops with large and waxy leaf surfaces grown in areas with ongoing or recent use of chlordane are more likely to contain elevated chlordane levels. In contrast, uptake of chlordane by roots is generally low due to its low water solubility. Kaul *et al.* (1972) determined the uptake of ^{14}C-labelled chlordane by carrots. The application was 0.19 mg ^{14}C-labelled *trans*-chlordane/kg soil. After 12 weeks carrot leaves, root surface and inner part of the carrot roots contained 0.88, 2.42 and 0.26 % of the applied radioactivity.

In most animal species, following uptake, chlordane is metabolised mainly to oxychlordane (see Chapter 6). Hence, different contamination patterns are found in feedingstuffs of plant and animal origin.

[20] See EMRL values at http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp

In the following paragraphs recent data received from Member States, EEA countries and stakeholders are summarised.

In Belgium a total of 870 single and compound feed samples were collected and analysed between 2000 and 2004. The analytical methods covered *cis*- and *trans*-chlordane, each at a given limit of quantification of 2 µg/kg. One sample of (SM: simple-mat.Prem./S) contained chlordane at 9 µg/kg. All other samples were negative.

Estonia reported on the results of 42 feed samples, mainly grain and complete feedingstuffs which were analysed between May 2004 and March 2005 for undesirable substances. Chlordane could not be detected in any sample at a limit of detection of 20 µg/kg.

In Denmark, 993 feed samples were analysed for undesirable substances between January 1998 and October 2004. Only 2 samples were found to be positive for chlordane. One animal fat sample contained 30 µg/kg and one complementary feedingstuff for cattle 4 µg/kg.

Finland recently provided data from analyses of undesirable substances in 14 feed samples from plant and animal origin. In all samples chlordane (expressed as sum of *cis*- and *trans*-chlordane and oxychlordane) was below the limit of determination. This limit of determination was 20 µg/kg except for one sample of cod liver oil, which had a limit of determination of 50 µg/kg, all expressed on the basis of 12 % moisture content.

Germany reported on the results of 290 feed samples of plant origin collected and analysed in 2004 for undesirable substances. None of these samples, which were mainly comprised of soy bean products, citrus pulp pellets, corn pellets and palm kernel derived products, contained chlordane above the limit of detection of 5 µg/kg.

Norway reported on the analyses of 33 feed samples for undesirable substances including *cis*- and *trans*-chlordane and oxychlordane in 2004. The samples covered commodities of plant origin, such as soybean meal, wheat grains and vegetable oils as well as specimens of animal origin, such as fish meal and fish oil. The limit of detection for *cis*-chlordane, *trans*-chlordane and oxychlordane were given as 0.5, 0.7 and 1.3 µg/kg, respectively. Ten samples were positive for *cis*-chlordane, 3 for *trans*-chlordane and 4 for oxychlordane. All samples were of marine origin. The highest levels were found in 4 fish oil samples with concentrations for *cis*-chlordane of 19, 19, 15 and 13 µg/kg. The corresponding levels for *trans*-chlordane and oxychlordane in these samples were 4.5, 3.6, 2.3, <0.7 and 2.9, 2.9, 3.0 and 3.4 µg/kg. Moreover, all samples were analysed for *cis*- and *trans*-nonachlor and a given limit of detection of 0.7 and 0.5 µg/kg. These compounds are minor constituents of technical chlordane. Eight samples were positive for *cis*-nonachlor and 10 samples for *trans*-nonachlor. As for chlordane, all positive samples were of marine origin. The concentration of *cis*-nonachlor and *trans*-nonachlor in the positive samples ranged from 1.9 – 9.2 and 0.8 – 20 µg/kg. The highest levels were found in fish oil. While fish oil was dominated by *trans*-nonachlor (ratio *trans*-nonachlor/*cis*-nonachlor = 2 – 4 : 1) fish meal and fish silage showed just the opposite ratio. It is noteworthy that the samples that are positive for chlordane

compounds in general contain higher levels of *cis*- and *trans*-nonachlor which indicates a higher persistency for these minor constituents of technical chlordane.

In 2003 and 2004 analyses for *cis*-chlordane, *trans*-chlordane and oxychlordane and other undesirable substances in 16 fish meal samples were performed in the Czech Republic. One sample contained *cis*-chlordane at a concentration of 1.09 µg/kg. All other samples were negative at a limit of detection of 1 µg/kg.

Iceland reported on the results of 23 fish meal and 17 fish oil samples analysed in 2003/2004 for organochlorine pesticides. The levels of chlordane expressed as the sum of *cis*-chlordane, *trans*-chlordane, oxychlordane and *trans*-nonachlor) in fish meal and fish oil ranged from 1.06 – 6.18 and 13 – 142.2 µg/kg, respectively. The highest levels were found in samples of blue whiting oil. Irrespective of the sample matrix, *cis*- and *trans*-chlordane, oxychlordane and *trans*-nonachlor amounted on average to the sum of the four chlordane compounds at 50, 12, 12 and 26 %.

The European Feed Manufacturers Federation (FEFAC) provided data for chlordane (expressed as sum of *cis*-chlordane, *trans*-chlordane and oxychlordane) on 11 fish feed and 13 fish oil samples. The chlordane levels in fish feed were in the range of 2 – 11 and in fish oil of <1 - 65 µg/kg.

Other data on nine fish feed samples for salmonids also provided by the FEFAC revealed in all cases positive results for *cis*-chlordane (0.53 – 5.9 µg/kg), *trans*-chlordane (0.46 – 1.57 µg/kg), oxychlordane (0.10 – 1.58 µg/kg), *cis*-nonachlor (0.21 – 3.91 µg/kg) and *trans*-nonachlor (0.44 – 8.64 µg/kg). In most of the samples the concentrations for the sum of the two nonachlor isomers exceeded the sum of *cis*-chlordane, *trans*-chlordane and oxychlordane.

Cis- and *trans*-chlordane, oxychlordane and *trans*-nonachlor were determined in the edible part of more than 140 fish samples of 15 different species and in fish meal, fish oil and fish feed (Karl *et al*., 1998). Highest concentrations were found in muscle of marine fish with high or moderate fat content, such as in eel and farmed salmon. Marine fish with low fat content contained only traces of chlordane in the muscle tissue. A relationship between fishing ground and levels of chlordane could not be established. Contamination level of herring was related to the age (length) of the fish. As also found by Karl *et al*. fish meal contained only small amounts of the compounds above, irrespectively of origin. Crude fish oil from Northern European production, made from various fish species, contained considerable amounts of sum of *cis*- and *trans*-chlordane and oxychlordane (10 – 77 µg/kg fat) and *trans*-nonachlor (5 - 79 µg/kg fat). Crude fish oil and fish meal from Chile only contained traces of *cis*-chlordane. In Antarctic krill oil none of the analysed chlordane compounds were detected at a LOD of 1 µg/kg. In both, trout and salmon feed, up to 4.1 µg total chlordane/kg w.w. and measurable amounts of *trans*-nonachlor were found by Karl *et al.* (1998).

In conclusion, the data on the occurrence of chlordane compounds including oxychlordane in feedingstuffs indicate that commodities of plant origin only occasionally show levels of these

compounds above the limit of detection. Higher levels can be found in specimens of animal origin, especially samples of marine origin. These samples also often show relatively high levels of *cis*- and *trans*-nonachlor, which represent only minor constituents of technical chlordane. However, the importance of these two compounds which seem to have a higher persistency than the major constituents of technical chlordane, such as *cis*- and *trans*-chlordane as well as their metabolite oxychlordane, is indicated by their generally higher levels compared to the latter compounds in fish derived products. This demonstrates the need to include at least *cis*- and *trans*-nonachlor into the analysis of feed samples, especially of animal origin for chlordane residues.

5. Adverse effects on fish, livestock and pets, and exposure-response relationship

Fish and terrestrial animals may be exposed to chlordane through contaminated diet. The compounds are most toxic in oil solutions particularly in vegetable oil. In addition, fish may be exposed through water and sediments, and livestock through dermal exposure. Thus, ingestion as well as absorption via gills or lungs, and skin are all possible portals of entry (Humphreys, 1988).

The sensitivity to chlordane exposure varies with species, strain, age, gender, health status and fat depot. Lean animals are more susceptible to acute intoxications than fat animals, since the insecticide is trapped in fat.

Acute intoxication is expressed through stimulation of the central nervous system. The symptoms vary considerably but are predominantly neuromuscular. The onset of clinical signs depends on the dose applied or ingested (Humphreys, 1988).

The signs of chronic toxicity are principally similar to those of acute intoxication and usually develop more gradually with tremors, convulsions, and depression which may last for weeks. Liver enlargement and necrosis are also common effects (Humphreys, 1988).

There has been a range of chlordane products with different composition and levels of impurities, which may have influenced the toxic potency of the products given (see also section 1.1.).

5.1. Fish

Acute toxic effects in various fish species exposed to chlordane via water for 96 hours were reported with LC_{50}s ranging from 7.8 to 500 µg/L (the latter value is above the water solubility, see 1.1.) (WHO, 1984). The rainbow trout (*Oncorhynchus mykiss)* showed the lowest LC_{50}. Fish showed a higher susceptibility to chlordane at higher water temperatures (Macek e*t al.,* 1969). Nutrition has also been shown to affect the chlordane toxicity in fish exposed via water, as rainbow trout fed a low protein diet (23 %) were more susceptible than

those fed a higher protein diet (45 %) (Mehrle *et al.,* 1974). Little *et al.* (1990) have shown that sublethal exposure of chlordane to rainbow trout via water (2 µg/L for 96 hours) influenced the behaviour after exposure. The exposure reduced the swimming and strike activities and the feed consumption.

No data on effects following dietary exposure to chlordane in fish have been identified.

5.2. Ruminants

Calves, 1 - 2 weeks old, received a single treatment of chlordane at 10, 25, 50 or 100 mg/kg b.w. Five calves given 10 mg/kg were unaffected by the treatment. Two calves were given 25 mg/kg, and one showed mild neurotoxic symptoms whereas symptoms were more severe in the other. Three out of four calves given 50 mg/kg were severely intoxicated within four hours and two of them died. No symptoms of the treatment were observed in the fourth calf. All three calves given 100 mg/kg were severely intoxicated within four hours and died (Radeleff *et al.,* 1955).

Welch (1948) found that a single oral dose of chlordane of 50 mg/kg b.w. administered to a steer induced no clinical effects. Marsh *et al.* (1951) reported the minimum toxic single oral dose of chlordane for cattle to be 91 mg/kg b.w. and the minimum lethal dose to be 129 mg/kg b.w.

An individual adult sheep was given a single dose of 50 mg/kg b.w. and did not elicit toxic symptoms. Of six adult sheep treated with a single dose of 100 mg/kg b.w., five were clinically intoxicated but none of them died (Radeleff *et al.,* 1955).

Welch (1948) found that a single oral dose of chlordane at 500 mg/kg b.w. to a sheep induced incoordination and partial blindness. Full clinical recovery occurred in 5 - 6 days. A dose of 1000 mg/kg b.w. to another sheep induced severe respiratory and nervous symptoms 16 hours after treatment and caused death after 48 hours.

Three adult goats were treated with chlordane in gelatine capsules at 750 mg/kg b.w. per day. Two of the animals received three doses and one received two doses before they died after neurotoxic effects and dyspnea (Choudhury and Robinson, 1950).

Used as single dermal whole body application, the minimum toxic concentration of chlordane for 1 - 2 week-old calves appeared to be between 1 and 2 % (suspension or emulsion). The minimum toxic single dermal dose for older cattle appeared to be above 2 % (Radeleff *et al.,* 1955).

5.3. Horses

No data were identified.

5.4. Pigs

Six pigs were fed a diet containing 300 mg/kg of either *cis*-chlordane or *trans*-chlordane for 30 - 90 days. They did not appear to be adversely affected, although enlarged livers of normal colour and texture were seen (Biotox, 1968, cited in NRCC, 1974).

5.5. Birds

From seven days of age, domestic chicks were fed chlordane at concentrations of 2500 or 5000 mg/kg in their feed (Rosenberg and Adler, 1950). Domestic chicks fed the highest concentration chirped nervously and those seriously afflicted were down on their hocks or on their sides within 24 hours and entered terminal agonal stages of excitability. All individuals in this group were dead within 78 hours. Chicks fed the lowest dose died within 96 hours.

Chlordane in various doses was given in the diet of growing domestic chickens of various ages (Rosenberg and Tanaka, 1950), i.e. from age 7 days, at 500, 1000, 1500, 2000 and 2500 mg/kg diet for up to 14 days, from age 21 days at 500, 1000 and 2500 mg/kg for 21 days, and at ages 63 and 112 days at 2500 and 5000 mg/kg for 21 days. In chickens fed chlordane from age 7 days, 500 mg/kg was lethal to 2/3 of the animals and higher doses were lethal to all within 14 days. Chickens of 21 days of age were slightly less susceptible to chlordane, however, 500 mg/kg was lethal to about 2/3 of the animals within 21 days and higher concentrations killed them all. For chicken 63 and 112 days of age, chlordane at 2500 mg/kg was lethal to almost all and 5000 mg/kg was lethal to all. The primary lesions noted in the study were reported to be in the heart.

Laying domestic pullets were fed a diet with chlordane at 500, 1500, 2500 or 5000 mg/kg for 28 days (Rosenberg *et al.,* 1950). All but the lowest dosage level significantly reduced the egg production and the body weight compared with control animals. Feed intake was reduced at all dosage levels.

Hens and cocks fed a diet containing up to 0.3 mg/kg of chlordane (time period not given) showed no intoxication symptoms, and no adverse effects on growth, hatchability of eggs or 28-day growth of chicks were observed (Biotox, 1969, cited in NRCC, 1974).

The oral LD_{50} of chlordane in young mallard (*Anas platyrhynchos)*, bobwhite quail (*Colinus virginianus*), Japanese quail (*Coturnix japonica*) and ring-necked pheasant (*Phasianus colchicus*) were 858, 331, 350 and 430 mg/kg diet, respectively, when fed for 5 days (Hill *et al.,* 1975).

The effect of chlordane on avoidance responses to a moving silhouette was studied in Coturnix quail chicks (Kreitzer and Heinz, 1974). The birds were fed chlordane at 25 mg/kg

diet from age 7 – 15 days (approximately 2.5 mg/kg b.w. per day) and the response was measured daily. The chlordane treatment suppressed the avoidance response significantly.

In birds fed purified oxychlordane or technical chlordane, oxychlordane was six times more toxic (Stickel *et al.*, 1979).

5.6. Rabbits

The oral LD_{50} of a single dose of Octachlor (a trade name of chlordane) to rabbits was approximately 300 mg/kg b.w. when dissolved in olive oil and approximately 100 mg/kg b.w. when dissolved in the detergent Tween 20. Octachlor dissolved in olive oil was also given to rabbits at daily doses of 5, 10, 15, 20, 50, 100 or 200 mg/kg b.w until death. At 5 mg/kg b.w. four of nine animals survived the maximum of 81 doses and the mean total dose for this group was >382 mg/kg b.w.. For the next dosage levels the mean cumulative doses until death were 188, 161, 172, 389, 567 and 967 mg/kg b.w., respectively, indicating a high cumulative action and higher relative absorption of lower doses. The exception was at 5 mg/kg b.w. where a balance between absorption and elimination may have been established. Urine examinations revealed development of albuminuria a few days before death. Pathological findings were focal coagulation necrosis and fatty and hyaline degenerations in the liver, degenerative changes in the proximal convoluted tubules in the kidneys, exudates in the respiratory tract, and frequent cellular infiltration of the intestinal submucosa (Stohlman *et al.,* 1950).

Chlordane was orally administered to pregnant rabbits at 1, 5 or 15 mg/kg b.w. per day from day 6 to 18 of gestation (IRDC, 1972). No changes were seen in behaviour, appearance or body weight. Miscarriages were seen in some individuals but not correlated with the dose level. No maternal or foetal effects including teratogenicity were noted.

5.7. Dogs

Dogs were given chlordane in a single oral dose up to 200 mg/kg b.w. before toxic symptoms were shown (Batte and Turk, 1948). No deaths were induced with doses up to and including 700 mg/kg b.w., although clonic spasms, tremors and convulsions were produced in some dogs at the higher doses.

Dogs given chlordane in oral doses of 5 - 80 mg/kg b.w. per day died within 25 days to 93 weeks (Lehman, 1952).

Groups of male and female dogs were fed dietary levels of chlordane at 0, 0.3, 3, 15 or 30 mg/kg for up to 2 years (Wazeter, 1967). Abnormalities in clinical liver function tests were seen in the 15 and 30 mg/kg groups. In animals selected for necropsy at the end of the first year, increased relative liver weight and associated hepatocellular changes were found at 30 mg/kg. At the end of the second year, a dose-related increase in relative liver weight was seen

at 15 and 30 mg/kg with non-dose-related hepatocellular changes. Liver biopsies after 1, 3 and 6 months of dogs fed 30 mg/kg showed hepatocellular changes at 6 months but not at 1 or 3 months. No adverse effects were seen on behaviour, appearance, survival, weight gain, blood picture or the results of periodic physical examinations. A NOAEL of 3 mg/kg diet corresponding to 0.075 mg/kg b.w per day could be derived from the study.

5.8 Cats

No toxicity studies on cats could be identified.

6. Toxicokinetics and tissue residues

6.1. Absorption

Chlordane is readily absorbed after oral exposure. The extent of *trans*-chlordane absorption in rat was estimated by Ohno *et al.* (1986) by comparing the areas under the curves from plasma (concentration versus time curves) following oral or intravenous dosing. Gastrointestinal absorption was estimated to be approximately 80 % of the administered dose, and this percentage did not vary significantly over the dose range tested (0.1 - 1.0 mg/kg b.w). Following oral administration of 1 mg *cis*-chlordane/kg b.w., peak blood levels in rats (81 ng/mL) and mice (113 ng/mL) were found after 2 and 8 hours, respectively (Ewing *et al.*, 1985).

Based on the radioactivity excreted in urine and on tissue residues, *trans*-chlordane absorption in rabbits fed ^{14}C-*trans*-chlordane were estimated to be at least 50 % of the ingested dose (Poonawalla and Korte, 1971). Similar values were determined from the data reported by Barnett and Dorough (1974) for rabbits fed for 2 days a diet with 25 mg/kg of HCS 3260 (a chlordane of approximately 95 % purity, with a *cis*- to *trans*-chlordane ratio of 3:1, see section 1.1.).

Although no specific studies were carried out to determine the intestinal absorption of chlordane in humans, the presence of chlordane residues in the adipose tissue, brain, liver and plasma of individuals after accidental ingestion indicates that the compound is absorbed from the gastrointestinal tract in humans.

6.2. Distribution

In rats, one day after a single oral dose (ranging from 0.05 to 1.0 mg/kg b.w.) of ^{14}C-*cis*-chlordane, or ^{14}C-*trans*-chlordane, or ^{14}C-HCS 3260 (see sect. 1.1. and 6.1), the greatest concentration of the radiolabel was found in adipose tissue, followed by liver, kidney, brain, and muscle (Barnett and Dorough, 1974). In all the cases, probably due to slower elimination in

females, radioactive residues in the adipose tissue were significantly higher in females than in males. In addition, treatment with *trans*-chlordane resulted in higher concentrations in the tissues than with *cis*-chlordane. In the same study, oral administration of HCS 3260 over a longer period of time (56 days) did not change the distribution pattern of radioactivity from that observed following a single dose exposure.

Ewing *et al.* (1985) determined tissue concentrations of chlordane in mice and rats treated orally with a single dose of ^{14}C-*cis*-chlordane (1 mg/kg b.w.). Peak concentrations in mice occurred 4 hours post-dosing and were 808, 1180, 349, 68, and 164 ng/g for fat, liver, kidney, brain, and muscle, respectively. Rats had peak tissue concentrations of 1239 and 729 ng/g for fat and kidney, respectively, at 4 hours and of 1959, 221, and 130 ng/g for liver, brain, and muscle, respectively, at 2 hours.

A single administration of a mixture (1:1) of *cis*- and *trans*-chlordane (20 mg/kg for each of the isomers) to male mice resulted in higher concentrations of *cis*-chlordane in the investigated tissues than of *trans*-chlordane (Satoh and Kikawa, 1992). At day one, *cis*-chlordane concentrations in muscle, liver, kidney, brain, and spleen were 1260, 377, 136, 56, and 34 µg/kg tissue, respectively, while *trans*-chlordane concentrations in these tissues were 766, 103, 82, 37, and 22 µg/kg, respectively. The concentrations of *cis*- and *trans*-chlordane were similar in blood (29 and 22 µg/L, respectively). However, in other experiments performed in rat (Barnett and Dorough, 1974) and rabbit (Balba and Saha, 1978) tissue levels were higher for the *trans*-isomers in animals given equal amounts of *trans*- and *cis*-chlordane, suggesting a faster excretion pattern and/or a more efficient biotransformation of the *cis* isomer in these species.

Residues in the fat of three Holstein cows, each given repeated daily doses of HCS 3260 (see sect. 1.1. and 6.1) in gelatin capsules for 60 days (equivalent to 1, 10, or 100 mg/kg feed, based on a daily feed consumption of 22.7 kg per animal) were studied by Dorough and Hemken (1973). At 1 mg/kg, the sum of c*is*- and *trans*-chlordane, oxychlordane and *trans*-nonachlor in the fat biopsies increased from 0.24 mg/kg adipose tissue after 30 days feeding to 0.47 mg/kg adipose tissue after 60 days. Similar trends were obtained from higher doses.

Chlordane concentrations of 0.005 - 0.137 mg/kg have been measured in human placenta (Al-Omar *et al.,* 1986) indicating that distribution to the foetus is possible. Chlordane has also been detected in human milk (see also Chapter 8). Within the National Report on Human Exposure to Environmental Chemicals, the CDC/USA reported results for chlordane (oxychlordane) – measured in blood for 1999 to 2000 and 2001 to 2002 at 44.9 and 49.7 ng/g respectively (US-CDC, 2005).

A major component of technical chlordane is *trans*-nonachlor which is frequently found to be the major chlordane residue in human tissues, but not in rats.

6.3. Metabolism

Four metabolic pathways have been proposed for the metabolism of chlordane in mammals (Nomeir and Hajjar, 1987): (a) hydroxylation to form 3-hydroxychlordane, followed by dehydration to form 1,2-dichlorochlordene, the postulated precursor of oxychlordane; (b) dehydrochlorination to form heptachlor with the subsequent formation of heptachlor epoxide and various hydroxylation products; (c) dechlorination to monochlorodihydrochlordene, and (d) replacement of chlorine atoms by hydroxyl groups resulting in the formation of mono-, di-, and trihydroxy- metabolites, which are excreted or further metabolized by conjugation with glucuronic acid (Figure 6).

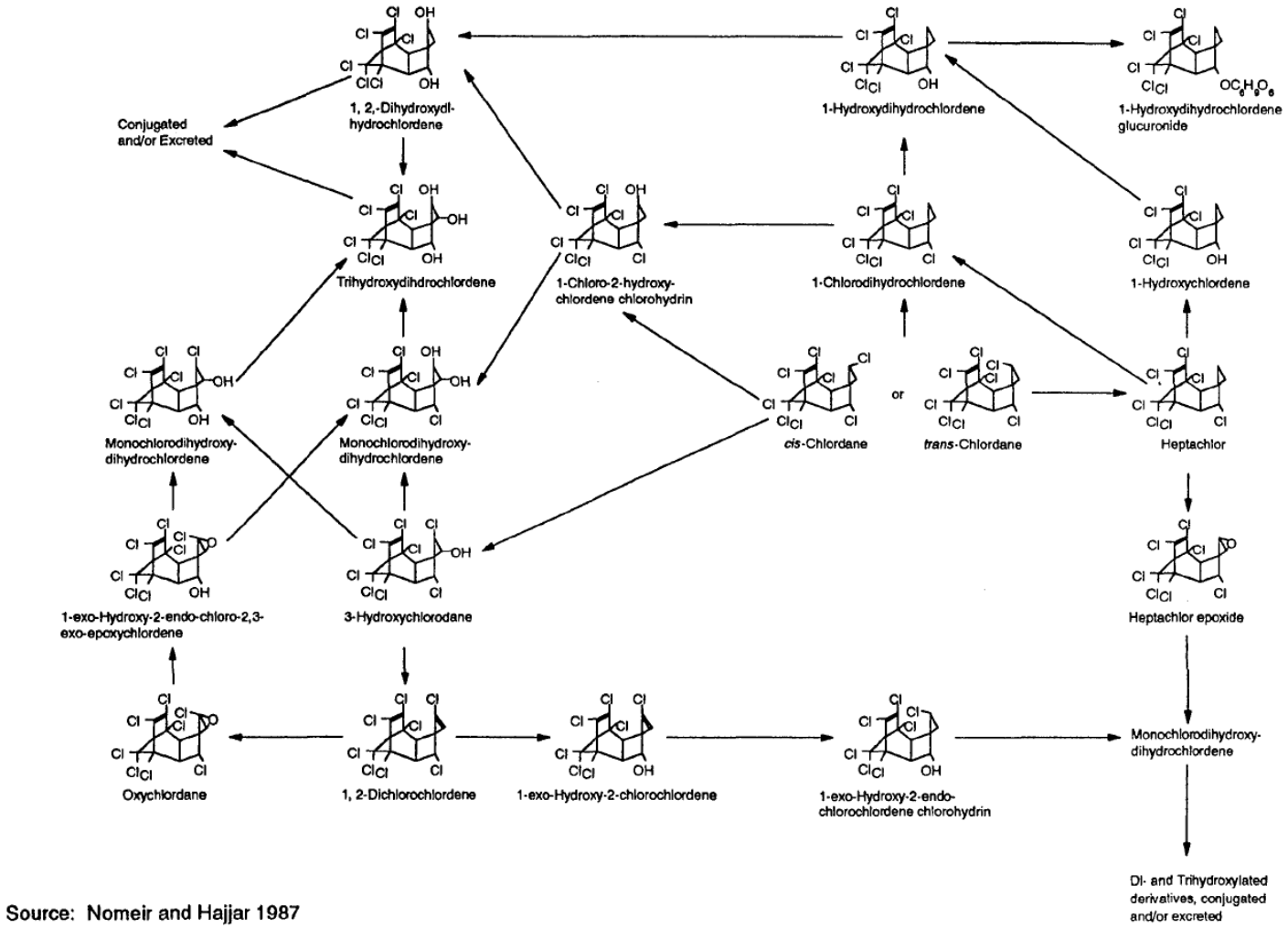


Figure 6. Metabolic pathways of chlordane in mammals

Oxychlordane was the major metabolite in all tissues of rats treated with ^{14}C-chlordane. Following a single oral dose of 1.0 mg/kg b.w. of HCS-3260 (see sect. 1.1. and 6.1), after 24 hours this metabolite accounted for 53 and 63 % of the radioactivity, in adipose tissue of females and males, respectively. Treatment with *trans*-chlordane resulted in greater percentages of oxychlordane in fat than did treatment with the *cis*- isomer. Analysis of liver and kidney from ^{14}C-HCS-3260 treated rats showed that 30 to 40 % of the radiolabelled residues was oxychlordane. Essentially all (>99 %) of the radioactivity in the fat of female rats treated with a single oral dose (0.2 mg/kg b.w.) of ^{14}C-oxychlordane was present as the parent compound (Barnett and Dorough, 1974).

Tashiro and Matsumura (1977) treated rats with a single oral dose of ^{14}C-*cis*- (5.4 mg/kg b.w.) or ^{14}C-*trans*-chlordane (9.7 mg/kg b.w.) and analysed the urine and faeces collected over a period of 7 days. Faecal extracts of *cis*-chlordane treated rats contained predominantly 1,2-dihydroxydihydroch1ordene (26.5 % of total radioactivity extracted from the faeces), 1–*exo*-hydroxy-2-chlorochlordene (19 %), monohydroxylated dihydrochlordene (15.5 %), *cis*-chlordane (13 %), and 1-*exo*-hydroxy-2-*endo*-chloro-2.3-*exo*-epoxychlordane (7.5 %). 1,2-Dichlorochlordene, 1–*exo*-hydroxy-2-*endo*-chlorodihydrochlordene (*i.e.* chlordane chlorohydrin), trihydroxydihydrochlordene, 1,2-dichlorochlordene, oxychlordane, heptachlor, and three unidentified metabolites were found in limited amount (each < 5 % of total radioactivity extracted from the faeces). Faecal extracts of *trans*-chlordane-treated rats contained principally l-*exo*hydroxy-2-chlorochlordene (29.5 % of total radioactivity extracted from the faeces), *trans*-chlordane (19 %), 1–exo-hydroxy-2-*endo*-chloro-2,3-*exo*-epoxy chlordene (14 %), 1,2-dihydroxydihydrochlordene (9.0 %), whereas chlordene chlorohydrin, monohydroxylated dihydrochlordenes, trihydroxydihydrochlordene, oxychlordane, 1,2-dichlorochlordene, heptachlor (0.1 %), and four unidentified compounds were minor metabolites. Urine and faeces from the ^{14}C-*cis*- or ^{14}C-*trans*-chlordane treated animals contained a glucuronide of 1-*exo*-hydroxydihydrochlordene. The glucuronide was the only metabolite identified in the urine. When ^{14}C-*cis*- or *trans*-chlordane was incubated with liver microsomes in the presence of various co-factors, 86 to 99 % of the radioactivity extracted with ether was oxychlordane, 1,2-dichlorchlordene, and heptachlor (Tashiro and Matsumura, 1978). Other metabolites identified were basically the same as those found in the *in vivo* studies.

The *in vivo* metabolism of *trans*-nonachlor in rats is similar to that of trans-chlordane (Tashiro and Matsumura, 1978). However, *in vitro* studies indicate that human liver microsomes have a much lower capacity to dechlorinate nonachlor to chlordane than rat liver microsomes (Tashiro and Matsumura, 1977).

Waterborne exposure of goldfish (*Carassius auratus*), bluegill (*Lepomis macrochirus*) and a cichlid belonging to the genus *Cichlasoma* to radiolabelled *cis*-chlordane revealed a very slow metabolism (Khan *et al.,* 1979). The cichlid resemble rats in the metabolic pathways of this compound by forming dichlorochlordene and oxychlordane, products which have not been detected in bluegill and goldfish. Chlordene chlorohydrin and hydroxylated biotransformation products were detected in all the three species.

The major metabolite of chlordane in humans is oxychlordane, which has been detected in the blood of pesticide applicators (Saito *et al.,* 1986). Dearth and Hites (1991a) determined that the nonachloro- and pentachlorocyclopentene (having 5 chlorine on cycle 2) components of chlordane were preferentially accumulated in human adipose tissue suggesting that people are unable to metabolize these isomers.

6.4 Excretion

Chlordane and its metabolites are mainly excreted in faeces of dosed rats. Approximately 85 to 90 % of chlordane given orally is excreted in the faeces within one week, whereas 2 to 8.6 % is found in the urine (Barnett and Dorough, 1974; Tashiro and Matsumura, 1977; Ewing *et al.,* 1985). Biliary excretion appears to be the major source of faecal excretion (Ewing *et al.,* 1985). These excretory patterns are similar to those occurring with the *cis*- and *trans*- isomers and with single and multiple dosing over a wide range of doses (0.05 - 9.7 mg/kg b.w. in single dose experiments or 1 to 25 mg/kg feed). The *cis*- isomer is excreted slightly faster than the *trans*-isomer. The excretory pattern of *cis*-chlordane in mice is similar to that in rats, except for a faster rate of elimination in mice during the first 14 hours (Ewing *et al.,* 1985). Learth and Hites (1991) measured the half-lives of depuration of 14 different chlordane components (e.g., *cis*- and *trans*-chlordane and *cis*- and *trans*-nonachlor) and metabolites (e.g., heptachlor epoxide, oxychlordane) from the fat of rats fed chlordane in the diet for 28 days. Half-lives ranged from 6 days (*cis*-chlordane) to 54 days (MC6).

The excretory pattern of chlordane in rabbits is different from that observed in mice and rats (Poonawalla and Korte, 1971; Barnett and Dorough, 1974; Balba and Saha, 1978). In rabbits, significantly more chlordane residues are excreted in urine (28 - 47 % of the dose). There are no major differences in the excretory pattern between *cis*- and *trans*-chlordane treated rabbits. Lactation is a substantial route of excretion of chlordane and related compounds (see Chapter 7).

Overall, metabolism and excretion of chlordane show a large interspecies difference.

7. Carry-over and tissue concentration

7.1 Transfer into milk and eggs

Chlordane (HCS 3260, purity of approximately 95 %, with a *cis*- to *trans*-chlordane ratio of 3: see Chapter 1.1. and 6.1) was administered to three Holstein cows in gelatine capsules after each morning milking for 60 days (Dorough and Hemken, 1973). The amounts of insecticide in the capsules were equivalent to those consumed by animals dietary exposed to 1, 10 or 100 mg HCS 3260/kg feed. At the 1.0 mg/kg feed level, chlordane residues expressed as the sum of *cis*- and *trans*-chlordane and oxychlordane in milk lipids reached a steady state of about 0.5 mg/kg lipid after 10 days, whereas approximately 35 and 45 days, respectively, were required

before a plateau was attained at approximately 2.5 mg/kg lipid and 5.0 mg/kg lipid when the cows were fed 10 and 100 mg of HCS 3260/kg diet, respectively. Oxychlordane accounted for 70 to 75 % of the sum of *cis*- and *trans*-chlordane and oxychlordane residues in milk, its relative concentration increasing with time. On the basis of the values reported for a cow exposed to 1 or 10 mg HCS 3260/kg feed, the transfer ratio (concentration in milk fat relative to the concentration in the diet) was between 0.25 and 0.43.

Chlordane feeding (80 µg/kg feed) for a one week period to laying hens (Herrick *et al.,* 1969), did not result in detectable residues in eggs (LOQ not given).

7.2 Tissue levels and bioaccumulation

In the study performed by Dorough and Hemken (1973) on Holstein cows dietary exposed to HCS 3260 (see Chapter 1.1,6.1 and 7.1), fat biopsies were taken from each animal 30 and 60 days after treatment was initiated. Oxychlordane accounted for 65 to 80 % of the sum of *cis*- and *trans*-chlordane and oxychlordane residues. The accumulation ratio of chlordane (concentration in tissues relative to the concentration in the diet) calculated for adipose tissue was in the 0.1 - 0.5 range.

The accumulation ratios in adipose tissue of pigs were reported to be between 0.3 and 0.9 (Noble, 1990).

Bioconcentration of chlordane from water to fish is well documented, but little is known about the bioaccumulation occurring through dietary exposure. Accumulation of chlordane in the liver and muscle of channel catfish (*Ictalurus punctatus*) dietary exposed for 28 days to technical chlordane (10, 20, 40 mg/kg feed) or to a 50:50 mixture of *cis*- and *trans*-chlordane (5, 10, 20, 40 mg/kg feed) was investigated by Murphy and Gooch (1995). The steady state concentration expressed as the sum of *cis*- and *trans*-chlordane and oxychlordane in muscle was reached after 3 weeks. The *cis* isomer accumulated preferentially to the *trans* isomer in both liver and muscle tissues, and the *cis* to *trans* ratio generally exceeded the corresponding ratio in the spiked feed. Oxychlordane was not detected in either tissue during any of the laboratory exposures. For both muscle and liver, the transfer ratio for *cis*- and *trans*-chlordane averaged each 0.04.

In a recent study, the transfer of chlordane from fish feed to rainbow trout was investigated (Karl *et al*., 2002). Trout were fed for 19 months a commercially available lipid-rich pelleted feed (fat content 26 – 30 %). The pellets were unspiked and *cis*- and *trans*-chlordane, oxychlordane and *trans*-nonachlor were measured in feed. The average concentrations were 3.8, 0.7, 1.7 and 4.3 µg/kg wet weight (corresponding to 13.3, 2.3, 5.9 and 15.0 µg/kg lipid) for *cis*- and *trans*-chlordane, oxychlordane, and *trans*-nonachlor respectively. No *trans*-chlordane and oxychlordane was found in muscle at the start of the experiment, whereas *cis*-chlordane concentration was below 0.1 µg/kg wet weight. Steady-state concentrations expressed as the sum of *cis*- and *trans*-chlordane, oxychlordane, and *trans*-nonachlor in

muscle lipids were reached after 6 months. Although the fat content of the fish increased dramatically during the experiment, thereafter, the chlordane concentrations expressed as the sum of *cis*- and *trans*-chlordane, oxychlordane and *trans*-nonachlor in the lipid did not change. After 6 months, the transfer rates given as a percentage ([concentration in edible part of the fish/total amount administered to fish during the period] x 100) were 14.2, 15.4, 19.4, and 16.7 for *cis*- and *trans*-chlordane, oxychlordane, and *trans*-nonachlor respectively. After 19 months, the percentages were 30.1, 24.7, 25.0, and 37.2 for these compounds respectively. The observed increase between months 6 and 19 is mainly related to the increase in lipid content from 2.6 % to 7.4 %. After 19 months, the transfer rates in female trout's were significantly lower than in male trout, indicating the importance of spawning for the elimination of chlordane in fish.

In fish, the carry-over varies according to the species and concentration of chlordane in feed. When current levels of feed contamination are tested for a long period of time (>6 months), a considerable amount of chlordane (about 40 % of the total doses administered via feed) can be transferred to the edible part of fish. Under these conditions, no major differences were observed between the chlordane compounds in terms of carry-over.

8. Human dietary exposure

Prior to the ban of chlordane, human exposure was mainly related to the application of chlordane as a pesticide in households and on farm land. Today food, particularly of animal origin is the primary source of chlordane exposure in the general population. This is because chlordane is no longer in use and because of the persistence and bioaccumulation of chlordane constituents and metabolites in the food chain.

Studies on recent dietary intake of chlordane are scarce. This is presumably due to the long-standing ban of this pesticide in most countries world-wide as well as the progressive decline of chlordane and its metabolites in humans as demonstrated by exposure assessments and analyses of human milk samples.

Total diet studies performed in the US between 1982/1984 and 1991 showed that daily dietary intakes of chlordane decreased from 5.1 to 1.3 ng/kg b.w. for infants, from 2 to 0.5 ng/kg b.w for teenagers, and from 2.7 to 1.5 ng/kg b.w. for adults (ATSDR, 1994). Intake studies performed across Canada between 1993 and 1998 resulted in average dietary intakes for the overall population (male and female) of 0.10 and 0.07 ng/kg b.w. per day for chlordane (Health Canada, 2003).

A total of 220 characteristic composite samples (representing an average food consumption basket) covering 205 food types in the form of 3696 individual samples were analysed in the Czech Republic in 2004/2005. Based on the results of these investigations the daily dietary chlordane exposure was calculated as 7 ng/kg b.w. for 4 - 6 year old children and 1.5 – 2.5 ng/kg b.w. for adults (Ruprich, 2006).

Assessments of dietary chlordane intake between 1970 and 1996 were performed in Poland by multiplying the mean annual consumption rates of food commodities by the residue concentration in the respective food items. Estimated dietary intakes of chlordane constituents were between 0.35 and 0.42 µg/person per day, equivalent to between 6 and 7 ng/kg b.w. per day for a 60 kg adult. The highest contribution was found to be from fish with dietary exposures of 0.09 – 0.13 µg/person per day, equivalent to 1.5 – 2 ng/kg b.w. per day for a 60 kg adult. A significant reduction in dietary chlordane exposure could not be observed between 1970 and 1996 (Falandysz, 2000, 2003).

Based on a market basket study performed in 1999, the dietary intake of chlordane and other organohalogen contaminants was assessed in Sweden. The estimated mean intake of chlordane (calculated as the sum of *cis*-chlordane, *trans*-chlordane, oxychlordane and *trans*-nonachlor) was found to be 115 ng/person per day (1.6 ng/kg b.w. per day based on the mean weight of 73.7 kg for the participants in the Swedish consumption study). Consumption of fish contributed 76 % to this intake followed by fats/oils, meat, dairy products, pastries, and eggs with contributions of 10, 5.4, 4.0, 2.6 and 1.6 %, respectively. This dietary chlordane exposure is considerably lower than in 1994 when a comparable assessment revealed a chlordane intake of 320 ng/person per day (4.3 ng/kg b.w. per day for a 60 kg adult) (Darnerud *et al*., 2006).

In the framework of the 3rd WHO human milk field study (Malisch *et al.,* 2004), *cis*-chlordane, *trans*-chlordane, oxychlordane and *trans*-nonachlor were analysed in 16 human milk pools from 10 European countries (Bulgaria, Czech Republic, Germany, Ireland, Italy, Luxembourg, Norway, Russia, Spain and Ukraine), and in 11 human milk pools from six non-European countries (Brazil, Egypt, Fiji, Hong Kong, Philippines and USA). While *cis*- and *trans*-chlordane were not detected at a limit of detection of 1 µg/kg milk fat, the concentration for oxychlordane and *trans*-nonachlor in all samples ranged from 1 – 18 (mean: 6.7; median: 6.0) µg/kg milk fat and <1 – 26 (mean: 7.5; median: 5.0) µg/kg milk fat, respectively. If only the European countries are considered, the oxychlordane and *trans*-nonachlor levels range from 3.5 -18 (mean: 7.2; median: 6.5) and 2.7 – 11 (mean: 5.8; median: 5.0) µg/kg milk fat. While the lowest levels in the European specimens were found in the pools from the Czech Republic and Ireland, the highest levels for oxychlordane was found in the pool from Bulgaria and for *trans*-nonachlor in the pools from Bulgaria and Norway.

Assuming an average daily intake of 800 ml breast milk with a fat content of 3.5 % for an exclusively breast fed baby weighing 5 kg, oxychlordane and *trans*-nonachlor concentrations of 6.5 and 5.0 µg/kg milk fat (median values for European countries of 3rd WHO human milk field study) would result in median daily intakes of around 36 and 28 ng/kg b.w., respectively.

In a comprehensive investigation of POPs pollution in the Asian regions Tanabe and co-workers (2007) collected breast milk samples of women from a number of Asian developing and developed countries between 1999 and 2003 and analysed them for chlordane constituents and other organochlorine compounds. The highest level for chlordane (sum of

oxychlordane, *cis*-nonachlor and *trans*-nonachlor was found in breast milk from Japan with a concentration of 110 µg/kg milk fat. The mean levels in the breast milk samples from India, Indonesia, Malaysia, Cambodia, Vietnam, Philippines and China ranged from 0.9 – 23 µg/kg milk fat. From the results it was concluded that the levels in breast milk of chlordane constituents in Asian developing countries are generally lower than in developed countries.

The few available data indicate that the average daily intake of chlordane is far below the PTDI of 500 ng/kg b.w. per day.

CONCLUSIONS

Production, use and environmental fate

- Chlordane was commercially introduced as a non-systemic contact insecticide in 1947. Technical chlordane consists of at least 147 constituents, 120 of which have been identified. The composition varies with the manufacturing process and during the period 1950 - 1970 the formulation consisted of 43 – 75 % *cis*- and *trans*-chlordane and lesser amounts of heptachlor, *cis*- and *trans*-nonachlor, and chlordenes. From the 1970s a more refined formulation containing more than 95 % *cis*- and *trans*-chlordane was also produced.

- Chlordane was used for agricultural purposes, mainly for soil and seed treatment and wood protection, the latter mostly in the USA. Chlordane has been banned for use in the European Union since 1981 and in most other countries world-wide.

- Chlordane is realatively stable in the environment and can be transported over long ranges in the atmosphere. *Cis*- and *trans*-chlordane, *cis*- and *trans*-nonachlor and also the metabolite oxychlordane tend to accumulate in the food chain. The half-life of chlordane in air, soil and water has been estimated to be in the range of months to many years. Congeners with higher numbers of chlorine atoms (≥8) have a higher persistency. Among the octachlorinated chlordane compounds, MC5 and U82 show the highest bioaccumulation factors.

General toxicological effects

- Chlordane shows moderate acute toxicity compared with other organochlorine pesticides. LD_{50}s for rats and mice are in the range of 335 - 430 mg/kg b.w.

- Oxychlordane, and to a lesser extent nonachlor, are more toxic than *cis*- and *trans*-chlordane. The main target organs are the nervous system and the liver. Chlordane causes liver tumours in mice probably via a non-genotoxic mechanism. Chlordane is classified by IARC as possibly carcinogenic to humans (group 2B). The WHO has

established a PTDI of 0.0005 mg/kg b.w. (500 ng kg/b.w.) based on liver toxicity in rats.

Adverse effects of chlordane in target animals

- No studies on adverse effects in fish following exposure to chlordane in feed have been identified. Chlordane shows variable toxicity to different fish species exposed via water (LC_{50}, 96 hours, 7.8 – 500 µg/L). Rainbow trout showed the lowest LC_{50} and a dose of 2 µg/L was sub lethal, but associated with signs of toxicity.
- In dogs, a NOAEL for chlordane of 3 mg/kg diet (corresponding to 0.075 mg/kg b.w.) was derived based on liver toxicity.
- For other species, no NOAEL or LOAEL could be derived from the available studies.

Contamination of feed

- Chlordane (sum of *cis*- and *trans*-chlordane and oxychlordane) is mostly found in fish derived products and only very infrequently in feed materials of plant origin or in terrestrial animals. Feed materials of marine origin may also contain *cis*- and *trans*-nonachlor at levels comparable with those of chlordane. However, the concentrations found are generally in the low µg/kg range and thus well below those that have been found to cause adverse effects in animals.

Fate in animals and carry-over

- Chlordane is readily absorbed after oral exposure and accumulates in lipid rich tissues. Oxychlordane is a major residue found in tissues of mammals. Compared with mammals, fish are less capable of forming oxychlordane, the major residues being *cis*-chlordane and *trans*-nonachlor. No metabolic data were identified for birds.
- The half-life of chlordane in rats varies from 6 (*cis*-chlordane) to 54 days (MC6) for the different constituents of technical chlordane. Due to the limited kinetic data no half-lives for other species could be calculated.
- In cow's milk, mainly oxychlordane was detected following chlordane exposure. The transfer ratio (concentration of chlordane, expressed as the sum of *cis*- and *trans*-chlordane and oxychlordane, in milk fat relative to the concentration in the diet) was in the range of 0.25 - 0.43 in dairy cows.
- Due to the poor quality of the data on laying hens, the transfer of chlordane into eggs can presently not be estimated.

- The accumulation ratio (concentration of chlordane, expressed as the sum of *cis*- and *trans*-chlordane and oxychlordane, in tissues relative to the concentration in the diet) in dairy cows and pigs varied from 0.1 to 0.9 for adipose tissue.
- In fish, limited data indicate variation in the transfer rates between species and with chlordane concentration in the diet. In rainbow trout exposed at the low µg/kg feed level, the transfer rates for *cis*- and *trans*-chlordane, oxychlordane and *trans*-nonachlor calculated as percentage in the edible part of the fish in relation to the total doses administered via feed varied between 25 and 37 %.

Human exposure

- Data from recent exposure studies in some countries where chlordane was formerly used indicate a lower dietary chlordane exposure compared with estimations performed 20 years ago.
- Food of marine animal origin is the main source of human exposure to chlordane. Recent data indicate a dietary intake for adults and children in the low ng/kg b.w. per day range which is two to three orders of magnitude below the PTDI of 500 ng/kg b.w.
- Median exposure to oxychlordane and *trans*-nonachlor of exclusively breastfed infants in the EU was recently estimated to be around 36 and 28 ng/kg b.w., respectively.

DATA NEEDS AND RECOMMENDATIONS

- Besides *cis*- and *trans*-chlordane and oxychlordane, the analyses of feed samples, especially of marine origin, should also include the determination of *cis*- and *trans*-nonachlor. Since MC5 and U82 are frequently found at the top of the marine food web and in human adipose tissues, they should also be included in the analytical scheme. Hence, standards for the latter two compounds need to be made commercially available.
- At low concentration of chlordane in biological samples, laboratories show large discrepancies in the performance in inter-comparison studies. Therefore improvement of the analytical methods is needed for environmental monitoring.
- The Members States are requested by the Commission to report the results of their monitoring programmes on undesirable substances in animal feed as compliant or non-compliant only without information of concentrations determined. The availability of detailed occurrence data concerning compounds and corresponding concentrations rather than condensed summary reports would be one prerequisite for an exposure assessment and identification of areas with an unusual high level of contamination. A European reporting system that facilitates these tasks should be set up.

- There are few toxicological data on target animal species. However, taking into consideration the long lasting ban of chlordane use and the low levels detected in animal feed, the Panel does not consider such data urgently needed.

REFERENCES

Altschuh, J., Brüggemann, R., Santl, H., Eichinger, G., and Piringer, O.G. 1999. Henry's law constants for a diverse set of organic chemicals: experimental determination and comparison of estimation methods. Chemosphere, 39: 1871-1887.

AMAP (Arctic Monitoring and Assessment Programme), 2004. AMAP Assessment 2002: Persistent Organic Pollutants in the Arctic. Oslo, Norway. xvi+310 pp.

Anderson, D.J. and Hites, R.A. 1988. Chlorinated pesticides in indoor air. Environmental Science and Technology 22: 717-720.

Atlas, E. and Giam, C.S. 1988. Ambient concentration and precipitation scavenging of atmospheric organic pollutants. Water, Air, and Soil Pollution 38: 19-36.

ATSDR (Agency for Toxic Substances and Disease Registry), 1994. Toxicological profile for chlordane. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service. http://www.atsdr.cdc.gov/toxprofiles/tp31.html

Batte, E.G. and Turk, R.D. 1948. Toxicity of some synthetic insecticides in dogs. J Econ Entomol 41: 102-103.

Bernhoft, A., Wiig, Ø. and Skaare, J.U. 1997. Organochlorines in polar bears (Ursus maritimus) at Svalbard. Environ Pollut 95(2):159-75.

Bidleman, T.F., Cotham, W.E., Addison, R.F. and Zinck, M.E. 1992. Organic contaminants in the northwest Atlantic atmosphere at Sable Island, Nova Scotia, 1988-89. Chemosphere 24: 1389-1412.

Bidleman, T.F., Jantunen, L.M.M., Helm, P.A., Brorström-Lundén, E. and Juntto, S. 2002. Chlordane enantiomers and temporal trends of chlordane isomers in Arctic air. Environ Sci Technol 36: 539-544.

Biotox, 1968. Unpublished report from Biotoxicological Research Associates for Vesicol Chem. Corp, Chicago, Illinois, USA. Sited in National Research Council Canada. 1974. Report No 14094. Chlordane: Its effects on Canadian ecosystems and its chemistry. 189 pp.

Biotox, 1969. Unpublished report from Biotoxicological Research Associates for Vesicol Chem. Corp, Chicago, Illinois, USA. Sited in National Research Council Canada. 1974. Report No 14094. Chlordane: Its effects on Canadian ecosystems and its chemistry. 189 pp.

Buchert, H., Class, T. and Ballschmitter, K. 1989. High resolution gas chromatography of technical chlordane with electron capture- and mass selective detection. Fresenius Z Anal Chem 333(3): 211-217.

Buser, H.-R. and Müller, M.D. 1992. Enantiomer separation of chlordane components and metabolites using chiral high-resolution gas chromatography and detection by mass spectrometry techniques. Anal Chem 64: 3168-3175.

334 Buser, H.-R., and Müller, M.D. 1993. Enantioselective determination of chlordane
335 components, metabolites, and photoconversion products in environmental samples using
336 chiral high-resolution gas chromatography and mass spectrometry. Environ Sci Technol
337 27: 1211-1220.
338 Buser, M.D. and Müller, H.-R. 1994. Identification of the (+)- and (-)-enantiomers of chiral
339 chlordane compounds using chiral high-performance liquid chromatography/chiroptical
340 detection and chiral high-resolution gas chromatography/mass spectrometry. Anal Chem
341 66: 2155-2162.
342 Carvalho, F.P., Villeneuve, J.-P. and Cattini, C. 1999. The determination of organochlorine
343 compounds and petroleum hydrocarbons in a seaweed sample: results of a worldwide
344 intercomparison exercise. Tr Anal Chem 18(11): 656-664.
345 CEN (European Committee for Standardisation), 2005. Animal feeding stuffs –
346 determination of pesticides and PCBs by GC/ECD and GC/MS. Working document TC
347 327 WI 00327022 + 00327023.
348 Cetin, B., Ozer, S., Sofuoglu, A. and Odabasi, M. 2006. Determination of Henry's law
349 constants of organochlorine pesticides in deionized and saline water as a function of
350 temperature. Atmos Environ 40: 4538-4546.
351 Choudhury, B. and Robinson, V.B. 1950. Clinical and pathological effects produced in goats
352 by the ingestion of toxic amounts of chlordane and toxaphene. Am J Vet Res 11: 50-57.
353 Christen, K. 1999. U.N. negotiations on POPs snag on malaria. Environ Sci Technol 33:
354 444A-445A.
355 Darnerud, P.O., Atuma, S., Aune, M., Bjerselius, R., Glynn, A., Petersson Grawé, K. and
356 Becker, W. 2006. Dietary intake estimations of organohalogen contaminants (dioxins,
357 PCB, PBDE and chlorinated pesticides, e.g. DDT) based on Swedish market basket data.
358 Food Chem Tox 44: 1597-1606.
359 Dearth, M.A. and Hites, R.A. 1990. Highly chlorinated dimethanofluorenes in technical
360 chlordane and in human adipose tissue. J Am Soc Mass Spectrom 1: 99-103.
361 Dearth, M.A. and Hites, R.A. 1991a. Complete analysis of technical chlordane using negative
362 ionization mass spectrometry. Environ Sci Technol 25: 245-254.
363 Dearth, M.A. and Hites, R.A. 1991b. Chlordane accumulation in people. Env Sci Technol 25:
364 1279-1285.
365 Dearth, M.A. and Hites, R.A. 1991c. Depuration rates of chlordane compounds from rat fat.
366 Env Sci Technol 25: 1128-1133.
367 de Boer, J. and Wells, D.E. 1997. Chlorobiphenyls and organochlorine pesticides in fish and
368 sediments-three years of QUASIMEME laboratory performance studies. Mar Poll Bull
369 35: 52-63.
370 de Boer, J. 1999. Capillary gas chromatography for the determination of halogenated micro-
371 contaminants. Review. J Chromatogr A 843: 179-198.
372 de Boer, J. and Law, R.J. 2003. Developmenmts in the use of chromatographic techniques in
373 marine laboratories for the determination of halogenated contaminants and polycyclic
374 aromatic hydrocarbons. Review. J Chromatogr A 1000: 223-251.
375 EFSA (European Food Safety Authority), 2007. Opinion of the Scientific Panel on
376 Contaminants in the Food Chain on a request from the European Commission related to
377 heptachlor as undesirable substance in animal feed, The EFSA Journal (2007) 478: 1-48
378 http://www.efsa.europa.eu/en/science/contam/contam_opinions/ej478_heptachlor.html

379 Falandysz, J. 2003. Wielkość spożycia chlordanu, heptachloru i epoksydu heptachloru w
380 Polsce (in Polish). Roczn. Pzh. 54(1): 49-64.
381 Falandysz, J. 2000. Wielkość spożycia chlordanu z żywnościa pochodzenia morskiego w
382 Polsce (in Polish). Rocz. Pzh. 51(3): 229-39.
383 Falandysz, J., Strandberg, L., Strandberg, B., Bergqvist, P-A. and Rappe, C. 2000.
384 Pozostałości chlordanu w rybach w zatoce Gdańskiej (in Polish). Roczn. Pzh. 51(2), 119 –
385 128
386 Goerke, H., Weber, K., Bornemann, H., Ramdohr, S. and Plötz, J. 2004. Increasing levels
387 and biomagnification of persistent organic pollutants (POPs) in Antarctic biota. Mar Poll
388 Bull 48: 295-302.
389 Hegeman, W.J.M. and Laane, R.W.P.M. 2002. Enantiomeric enrichment of chiral pesticides
390 in the environment. Rev Environ Contam Toxicol 173: 85-116.
391 Health Canada. 2003. Average dietary intakes (ng/kg bw/day) of pesticide residues for
392 Canadians in different age-sex groups from the 1993 to 1996 Total Diet Study.
393 http://www.hc-sc.gc.ca/fn-an/surveill/total-diet/intake-apport/pesticide_intake-
394 apport_pesticide_93-96_e.html
395 Hill, E.F., Heath, R.G., Spann, J.W. and Williams, J.D. 1975. Lethal dietary toxicities of
396 environmental pollutants to birds. Special Scientific Report No. 191. Washington DC, US
397 Department of the Interior, Fish and Wildlife Service, 61 pp.
398 Hoekstra, P.F., O'Hara, T.M., Karlsson, H., Solomon, K.R. and Muir, D.C.G. 2003a.
399 Enantiomer-specific biomagnification of alpha-hexachlorocyclohexane and selected chiral
400 chlordane-related compounds wthin an arctic marine food web. Environ Toxicol Chem 22:
401 2482-2491.
402 Hoekstra, P.F., Braune, B.M., Wong, C.S., Williamson, M. Elkin, B. and Muir, D.C.G.
403 2003b. Profile of persistent chlorinated contaminants, including selected chiral
404 compounds, in wolverine (Gulo gulo) livers from the Canadian Arctic. Chemosphere 53:
405 551-560.
406 Hop, H., Borgå, K., Gabrielsen, G.W., Kleivane, L. and Skaare, J.U. 2002. Food web
407 magnification of persistent organic pollutants in poikilotherms and homeotherms from the
408 Barens Sea. Environ Sci Technol 36: 2589-2597.
409 HSDB (Hazardous Substances Data Bank), 2003. Oxychlorane. Updated 14.02.2003
410 TOXNET, Toxicology Data Network. http://toxnet.nlm.nih.gov/cgi-
411 bin/sis/search/f?./temp/~P6B9Pc:3
412 HSDB (Hazardous Substances Data Bank), 2005. Nonachlor. Updated 31.01.2005 TOXNET,
413 Toxicology Data Network. http://toxnet.nlm.nih.gov/cgi-
414 bin/sis/search/f?./temp/~P6B9Pc:2
415 Humphreys, D.J. 1988. Chlorinated hydrocarbon insecticides. In Humphreys D.J. Veterinary
416 Toxicology. 3. ed. Bailliere Tindall, London, p. 142-156.
417 IARC (International Agency on Research on Cancer), 2001. Monographs on the Evaluation of
418 the Carcinogenic Risk of Chemicals to Man. Geneva: World Health Organization,
419 International Agency for Research on Cancer, 1972-present. (Multivolume work), p. V79:
420 420.
421 IRDC (International Research and Development Corporation), 1972. Chlordane teratology
422 study in rabbits. Mattawan, Michigan, (Report 163-106, sponsored by Velsicol Chemical
423 Corporation. Referred in WHO, 1984.

Irvie, G.W. 1973. Nature and toxicity of two oxychlordane photoisomers. J Agric Food Chem 21: 1113-1115.

Iwata, H., Tanabe, S., Sakai, N. and Tatsukawa, R. 1993. Distribution of persistent organochlorines in the oceanic air and surface seawater and the role of ocean on their global transport and fate. Environ. Sci. Technol. 27: 1080 – 1098.

Jantunen, L. and Bidleman, T.F. 2006. Henry's law constants for hexachlorobenzene, p,p'-DDE and components of technical chlordane and estimates of gas exchange for Lake Ontario. Chemosphere 62: 1689-1696.

Jaward, F.M., Zhang, G., Nam, J.J., Sweetman, A.J., Obbard, J.P., Kobara, Y. and Jones, K.C. 2005. Environ Sci Technol 39: 8638-8645.

Kallenborn, R., Oehme, M., Wynn-Williams, D.D., Schlabach, M. and Harris, J. 1998. Ambient air levels and atmospheric long-range transport of persistent organochlorines to Signy Island, Antarctica. Sci Tot Environ 220: 167-180.

Karl, H., Lehmann I. and Oetjen K. 1998. Levels of chlordane compounds in fish muscle, -meal, -oil and -feed. Chemosphere 36(13): 2819-32.

Karl H., Lehmann I. and Oetjen K. 2002. Transfer of Toxaphene and Chlordane into farmed rainbow trout via feed. Aquaculture Research 33(12): 925-932.

Karlsson, H., Oehme, M. and Scherer, G. 1999. Isolation of the chlordane compounds U82, MC5, MC7, and MC8 from technical chlordane by HPLC including structure elucidation of U82 and determination of ECD and NICI-MS response factors. Environ Sci Technol 33: 1353-1358.

Karlsson, H., Oehme, M., Skopp, S. and Burkow, I.C. 2000. Enantiomer ratios of chlordane congeners are gender specific in cod (Gadus morhua) from the Barents Sea. Environ Sci Technol 34: 2126-2130.

Kaul, R., Weisgerber, I. and Klein, W. 1972: Verteilung und Umwandlung von trans-Chlordan-14C in Weißkohl und Möhren. Chemosphere, 2, 79-82.

Kearns, C.W. Ingle L. and Metcalf, R.L. 1945. New chlorinated-hydrocarbon insecticide. J Econ Entomol 38: 661.

Khan, M.A.Q., Feroz, M. and Sudershan, P. 1979. Metabolism of cyclodiene insecticides by fish. In Khan, M.A.Q., Lech, J.J. and Menn, J.J. (ed.): Pesticide and xenobiotic metabolism in aquatic organisms. Amer Chem Soc, Washington, D.C., USA.

Kirk, R.E. and Othmer, D.F. 1981. Concise Encyclopedia of Chemical Technology. Third edition, volume 13. John Wiley & Sons, Inc.

Knap, A.H. and Binkley, K.S. 1991. Chlorinated organic compounds in the troposphere over the Western North Atlantic Ocean measured by aircraft. Atmos Environ 25A: 1507-1516.

Kreitzer, J.F. and Heinz, G.H. 1974. The effect of sublethal dosages of five pesticides and apolychlorinated biphenyl on the avoidance response of coturnix quail chicks. Environ Pollut 6: 21-29.

Klobes, U., Vetter, W., Luckas, B., Skírnisson, K., and Plötz 1998. Levels and enantiomeric ratios of α-HCH, oxychlordane, and PCB 149 in blubber of harbour seals (Phoca vitulina) and grey seals (Halichoerus grypus) from Iceland and further species. Chemosphere 37: 2501-2512.

Lebeuf, M., Noël, M., Trottier, S. and Measures, L. 2007. Temporal trends (1987-2002) of persistent, bioaccumulative and toxic (PBT) chemicals in beluga whales (*Delphinapterus leucas*) from the St. Lawrence Estuary, Canada. Sci Tot Env 383: 216-231.

469 Lehman, A.J. 1952. A report to the Association of Food and Drug Officials on Current
470 Developments. Section, III: Subacute and chronic toxicity. Assoc Food Drug Off US Q
471 Bull 16: 47-53.
472 Little, E.E., Archeski, R.D., Flerov, B.A. and Kozlovskaya, V.I. 1990. Behavioral indicators
473 of sublethal toxicity in rainbow trout. Arch Environ Contam Toxicol 19: 380-385.
474 Macek, K.J., Hutchinson, C. and Cope, O.B. 1969. The effects of temperature on the
475 susceptibility of bluegills and rainbow trout to selected pesticides. Bull Environ Contam
476 Toxicol 4: 174-183.
477 Magnussen, M.P. and Vang, G. 2006. Accumulation of Dioxin, PCB and other Contaminants
478 in Farmed Salmon. Food, Veterinary and Environmental Agency, Tórshavn, Faroe Islands
479 and P/F Havsbrún, Fuglafjørður, Faroe Islands.
480 http://www.hfs.fo/pls/portal/docs/page/hfs/www_hfs_fo/umsiting/kunnanditilfar/kunnandit
481 ilfarritg/mpm-rapport.pdf
482 Malisch, R., Kypke, K., van Leeuwen, R. and Moy, G. 2004. Unpublished data from the 3rd
483 WHO human milk field study.
484 Marsh, H., Johnson, L.H., Clark, R.S. and Pepper, J.H. 1951. Toxicity to cattle of toxaphene
485 and chlordane grasshopper baits. Montana Agric Exp Sta Bull 477, 11 p.p.
486 Mehrle, P.M., Johnson, W.W. and Mayer, F.L. Jr. 1974. Nutritional effects on chordane
487 toxicity in rainbow trout. Bull Environ Contam Toxicol 12: 513-517.
488 Meylan, W.M. and Howard, P.H. 1991. Bond contribution method for estimating Henry's
489 law constants. Environ Toxicol Chem 10: 1283-1293.
490 Minh, T.B., Watanabe, M., Tanabe, S., Yamada, T., Hata, J. and Watanabe, S. 2002. Specific
491 accumulation and elimination kinetics of tris(4-chlorophenyl)methane, tris(4-
492 chlorophenyl)methanol, and other persistent organochlorines in humans from Japan.
493 Environ Health Persp 109: 927-302.
494 Miyazaki, T., Yamagishi, T. and Matsumoto, M. 1985. Isolation and structure elucidation of
495 some components in technical grade chlordane. Arch Environ Contam Toxicol 14: 475-
496 483.
497 Mongomery, J.H. 1997. Agrochemicals Desk Reference. Environmental Data. Lewis
498 Publishers, Chelsea, MI.
499 Muir, D. and Sverko, E. 2006. Analytical methods for PCBs and organochlorine pesticides in
500 environmental monitoring and surveillance: a critical appraisal. Anal Bioanal Chem 386:
501 769-789.
502 Muir, D.C.G., Norstrom, R.J. and Simon, M. 1988. Organochlorine contaminants in Arctic
503 marine food chains: accumulation of specific polychlorinated biphenyls and chlordane-
504 related compounds. Environ Sci Technol 22: 1071-1079.
505 Nomeir, A.A. and Hajjar, N.P. 1987. Metabolism of chlordane in mammals. Reviews of
506 Environmental Contamination and Toxicology 100: 1-22.
507 Norström, R.J., Simon, M., Muir, D.C.G. and Schweinsburg, R.E. 1988. Organochlorine
508 contaminants in Arctic marine food chains: identification, geographical distribution, and
509 temporal trends in polar bears. Environ Sci Technol 22: 1063-1071.
510 NRCC (National Research Council Canada), 1974. Report No 14094. Chlordane: Its effects
511 on Canadian ecosystems and its chemistry. 189 pp.

512 Patton, G.W., Walla, M.D., Bidleman, T.F. and Barrie, L. A. 1991. Polycyclic Aromatic and
513 Organochlorine Compounds in the Atmosphere of Northern Ellesmere Island, Canada, J
514 Geophys Res 96 (D6): 10 867-10 877.
515 Pozo, K., Harner, T., Wania, F., Muir, D.C.G., Jones, K.C. and Barrie, L.A. 2006. Toward a
516 global network for persistent organic pollutants in air: results from the GAPS study.
517 Environ Sci Technol 40: 4867-4873.
518 Primbs, T., Simonich, S., Schmedding, D., Wilson, G., Jaffe, D., Takami, A., Kato, S.,
519 Hatakeyama, S. and Kajii, Y. 2007. Atmospheric outflow of anthropogenic semivolatile
520 organic comounds from East Asia in spring 2004. Environ Sci Technol 41: 3551-3558.
521 Radeleff, R.D., Woodart, G.T., Nickerson, W.J. and Bushland, R.C. 1955. The acute toxicity
522 of chlorinated hydrocarbons and organic phosphorus insecticides to livestock. US Dept
523 Agric Tech Bull 1122: 1-46.
524 Rosenberg, M.M. and Adler, H.E. 1950. Comparative toxicity of DDT and chlordane to
525 young chicks. Am J Vet Res 11: 142-144.
526 Rosenberg, M.M. and Tanaka, T. 1950. Toxicity of chlordane to growing chickens. Am J Vet
527 Res 11: 233-235.
528 Rosenberg, M.M., Tanaka, T. and Adler, H.E. 1950. Toxicity of chlordane to laying pullets.
529 Am J Vet Res 11: 236-239.
530 Ruprich, J. 2006. Chlordane dietary exposure in the Czech Republic 2002-2004/5.
531 http://www.chpr.szu.cz/monitor/tds05c/CHEMON/org/CHLORDAN.pdf.
532 Shen, L. and Wania, F. 2005. Compilation, evaluation, and selection of physical-chemical
533 property data for organochlorine pesticides. J Chem Eng Data 50: 742–768.
534 Simpson, C.D., Wilkins, A.L., Langdon, A.G. and Wilcock, R.J. 1996. Chlordane residues in
535 marine biota and sediment from an intertidal sandbank in Manukau harbour, New
536 Zealand. Mar Poll Bull 32: 499-503.
537 Simpson, C.D., Wilcock, R.J., Smith, T.J., Wilkins, A.L. and Langdon, A.G. 1995.
538 Determination of octanol–water partition coefficients for the major components of
539 technical chlordane. Bull Environ Contam Toxicol 55: 149–153.
540 Sinkkonen, S. and Paasivirta, J. 2000. Polychlorinated organic compounds in the Arctic cod
541 liver: trends and profiles. Chemosphere 40: 619-626.
542 Stickel, L.F., Stickel, W.H., McArthur, R.D. and Hughes, D.L. 1979. Chlordane in birds: a
543 study of lethal residues and loss rates, p. 387-396. In Deichmann, W.B. [ed.]. Toxicology
544 and occupational medicine. Elsevier, North Holland, NY.
545 Stohlman, E.F., Thorp, W.T.S. and Smith, M.I. 1950. Toxic action of chlordan. Arch Ind Hyg
546 Occup Med 1: 13-19.
547 Strandberg, B., Strandberg, L., Bergqvist, P.A., Falandsz, J. and Rappe, C. 1998.
548 Concentrations and biomagnification of 17 chlordane compounds and other
549 organochlorines in harbour porpoise (Phocoena phocoena) and herring from the southern
550 Baltic Sea. Chemosphere 37: 2513-2523.
551 Sydney, A., Shain, J., Shaeffer, C. and Boesel, R.W. 1977. Toxicology and Applied
552 Pharmacology Volume 40, Issue 1, April 1977, Pages 115-130
553 Tanabe, S. and Kunisue, T. 2007. Persistent organic pollutants in human breast milk from
554 Asian countries. Environmental pollution 146: 400-413.
555 UNEP (United Nations Environment Programme) Chemicals 2002. Masterlist of actions on
556 the Reduction and/or Elimination of the Releases of Persistent Organic Pollutants. 4th

edition. UNEP Chemicals Geneva, Switzerland. http://www.pops.int/documents/meetings/inc6/englishonly/Masterlist4.pdf

US-CDC (U.S. Centers for Disease Control and Prevention), 2005. Third National Report on Human Exposure to Environmental Chemicals. Atlanta, GA. http://www.cdc.gov/exposurereport/pdf/thirdreport.pdf

US-EPA (U.S. Environmental Protection Agency), 1990. Nonoccupational pesticide exposure study (NOPES). U.S. Environmental Protection Agency, Research Triangle Park, NC. EPA 600/3-90-003. NTIS no. PB90-152224.

Vetter, W. and Schurig, V. 1997. Enantioselective determination of chiral organochlorine compounds in biota by gas chromatography on modified cyclodextrins. Review. J Chromatogr A 774: 143-175.

Villeneuve, J.-P., de Mora, S. and Cattini, C. 2004. Determination of organochlorinated compounds and petroleum hydrocarbons in fish-homogenate sample IAEA-406: results from a worldwide interlaboratory study. Tr Anal Chem 23(7): 501-510.

Warner, N.A. and Wong, C.S. 2006. The freshwater invertebrate Mysis relicta can eliminate chiral organochlorine compounds enantioselectively. Environ Sci Technol 40: 4158-4164.

Wazeter, F.X. 1967. Two-year chronic feeding study in the beagle dog. Mattawan, Michigan, International Research and Development Corporation (Report for the Velsicol Chemical Corporation) Referred in WHO, 1984.

Welch, H. 1948. Tests of the toxicity to sheep and cattle of certain of the newer insecticides. J Econ Entomol 41: 36-39.

Whetstone, R.R. 1964. Chlorocarbons and chlorohydrocarbons. In: Standen A, ed. Kirk-Othmer encyclopedia of chemical technology, 2nd ed, vol. 5. New York, NY: John Wiley and Sons, 248-249.

Wiberg, K., Oehme, M., Haglund, P., Karlsson, H., Olsson, M. and Rappe, C. 1998. Enantioselective analysis of organochlorine pesticides in herring and seal from the Swedish marine environment. Mar Poll Bull 36: 345-353.

Wiberg, K., Letcher, R.J., Sandau, C.D. Norstrom, R.J., Tysklind, M. and Bidleman, T.F. 2000. The enantioselevctive bioaccumulation of chiral chlordane and α-HCH contaminants in the polar bear food chain. Environ Sci Technol 34: 2668-2674.

Wong, C.S., Hoekstra, P.F., Karlsson, H., Backus, S.M., Mabury, S.A. and Muir, D.C.G. 2002. Enantimer fractions of chiral organochlorine pesticides and polychlorinated biphenyls in standard and certified reference materials. Chemosphere 49: 1339-1347.

Worthing, C.R. and Walker, S.B. eds. 1987. The pesticide manual: A world compendium. 8th ed. Suffolk, Great Britain: The British Crop Protection Council, 455-456.

Xu, D., Zhong, W., Deng, L., Chai, Z. and Mao, X., 2004. Organohalogenated compounds in pine needles from Beijing city, China. Chemosphere 57: 1343-1353.

Zhu, J, Norstrom, R.J., Muir, D.C.G., Ferron, L.A., Weber, J.-P. and Dewailly, E. 1995. Persistent chlorinated cyclodiene compounds in ringed seal blubber, polar bear fat, and human plasma from Notheren Québec, Canada: identification and concentrations of photoheptachlor. Environ Sci Technol 29: 267-271.

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