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# OPINION OF THE SCIENTIFIC PANEL ON CONTAMINANTS IN THE FOOD CHAIN ON A REQUEST FROM THE EUROPEAN COMMISSION RELATED TO HEPTACHLOR

## AS AN UNDESIRABLE SUBSTANCE IN ANIMAL FEED

**Question N° EFSA-Q-2005-184**

Adopted on 26 April 2007

## SUMMARY

Heptachlor was commercially introduced as a non-systemic contact insecticide in 1945. It was also a major constituent (about 10 %) of technical chlordane. Heptachlor was used for agricultural purposes, soil and seed treatment, wood protection and termite- and household insect control. It has been banned for use in the European Union since 1984 and in most other countries worldwide because of the persistency in the environment of the two break-down products heptachlor epoxide and photoheptachlor. All these compounds are lipophilic and particularly heptachlor epoxide and photoheptachlor tend to accumulate in the food chain.

Heptachlor shows moderate acute toxicity and heptachlor epoxide and photoheptachlor are more toxic than heptachlor. In mammals, the main target organs are the nervous system and the liver, but also the reproductive and the immune system are affected. Heptachlor and heptachlor epoxide cause liver tumours in mice, but are not genotoxic. Heptachlor is classified by IARC as possibly carcinogenic to humans (group 2B). Heptachlor is moderately or highly toxic to fish exposed via water, but no data from oral studies have been found.

Amongst the species studied, the domestic hen is the most sensitive species and egg production and hatchability are the critical endpoints. Total heptachlor (sum of heptachlor and heptachlor epoxide) is not frequently found in feed commodities. When present, it is mostly in fish derived products and only very infrequently in feed materials of plant origin. Heptachlor epoxide is the predominant contaminant. The concentrations found in feed are in the low µg/kg range and thus well below those that have been found to cause adverse effects in animals. The half-life of total heptachlor varies from several days in rodents up to more than 20 weeks in non-lactating cattle. Following heptachlor exposure, only heptachlor epoxide is found in milk and eggs. The present dietary exposure of the adult population to total

heptachlor is below 1 ng/kg b.w. per day, which is two to three orders of magnitude below the tolerable daily intake of 0.0001 mg/kg b.w. as established by WHO in 2006.

**KEYWORDS:** Heptachlor, heptachlor epoxide, photoheptachlor, persistence, insecticide, animal feed, toxicity, analysis, occurrence, metabolism, bioaccumulation, animal health, carry-over, human health.

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## LIST OF ABBREVIATIONS AND ACRONYMS

| | |
|---|---|
| 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 |
| $LD_{50}$ | 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 |
| MAPKS | Mitogen-activated protein kinases |
| ML | Maximum level |
| MRL | Maximum residue level |
| MS | Mass spectrometry |
| NADP | Nicotinamide adenine dinucleotide phosphate |
| 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

## 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[1] replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition[2].

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 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 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)[3].

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003[4] provides a comprehensive overview on the possible risks for

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

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

Heptachlor is a man made chemical and does not occur naturally. Heptachlor has been used as insecticide to control termites in houses and insects in agriculture.

The use of heptachlor as a pesticide has been very restricted sine 1981 and has been banned since October 1984 in the EU by Council Directive 79/117/EEC of 21 December 1978[5] 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

- Directive 76/895/EEC of 23 November 1976 relating to the fixing of maximum levels for pesticide residues in and on fruit and vegetables[6]
- Directive 86/362/EEC of 24 July 1986 on the fixing of maximum residue levels for pesticide residues in and on cereals[7]
- 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[8]
- 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[9].

Until 1997, MRLs were fixed only for raw commodities. Council Directive 1997/41/EC of 25 June 1997[10] 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

[5] OJ L 33, 8.2.1979, p. 36
[6] OJ L 340, 9.12.1976, p.26
[7] OJ L 221, 7.8.1986, p. 37
[8] OJ L 221, 7.8.1986, p. 43
[9] OJ L 350, 14.12.1990, p. 71
[10] OJ L 184, 12/07/1997, p. 33

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[11] 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.

Heptachlor (sum of heptachlor and of heptachlor epoxide, expressed as heptachlor) is listed in the Annex to Directive 2002/32/EC.

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

| **Directive 2002/32/EC** | |
|---|---|
| ML for heptachlor (sum of heptachlor and of heptachlor epoxide, expressed as heptachlor) relative to a feedingstuff with a moisture content of 12 % | |
| **Product** | **mg/kg** |
| Fats | 0.2 |
| Other feedingstuffs | 0.01 |
| | |

| **EU-Pesticide residue legislation** | |
|---|---|
| MRL for heptachlor (sum of heptachlor and of heptachlor epoxide, expressed as heptachlor) applicable to the product as marketed | |
| **Product** | **mg/kg** |
| Fruit and vegetables | 0.01 |
| Oilseeds | 0.01 |
| Cereals | 0.01 |
| Meat (fat) | 0.2 |
| Milk | 0.004 |
| Eggs | 0.02 |

The maximum levels for heptachlor in Directive 2002/32/EC are comparable to those in the pesticide legislation.

[11] OJ L 70, 16.3.2005, p. 1

## TERMS OF REFERENCE

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 heptachlor in animal feed.

This scientific opinion should comprise the

- determination of the toxic exposure levels (daily exposure) of heptachlor 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 heptachlor from the feed to the products of animal origin results in unacceptable levels of heptachlor 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 heptachlor 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 heptachlor
    - to the overall exposure of the different relevant animal species to heptachlor,
    - 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.

## ASSESSMENT

### 1. Introduction

Heptachlor is a persistent chlorinated insecticide, which was extensively used from 1953 to 1974 for soil and seed treatment and to control termites and household insects. As a result of its bioavailability and persistence, it is now banned in most countries including the EU and USA. In the environment, heptachlor is mainly converted to heptachlor epoxide and photoheptachlor, which are both persistent. Unless otherwise stated the term total heptachlor refers to the sum of heptachlor and heptachlor epoxide.

## 1.1. Synthesis and chemistry

Heptachlor (1,4,5,6,7,8,8a-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene; CAS No. 76-44-8; $C_{10}H_5Cl_7$) was first identified in 1946 as a constituent of technical chlordane of which heptachlor constitutes about 10 % (Buchert *et al.,* 1989; Buser and Müller, 1993; NRCC, 1974). Heptachlor has a 3 - 5 times greater insecticidal activity than technical chlordane and was later used as an insecticide on its own (Kirk-Othmer, 1981). Heptachlor is formed by a reaction of hexachlorocyclopentadiene with cyclopentadiene with chlordene as an intermediate which is further chlorinated (with chlorine in the dark in the presence of Fuller's earth) to technical heptachlor, see Figure 1.

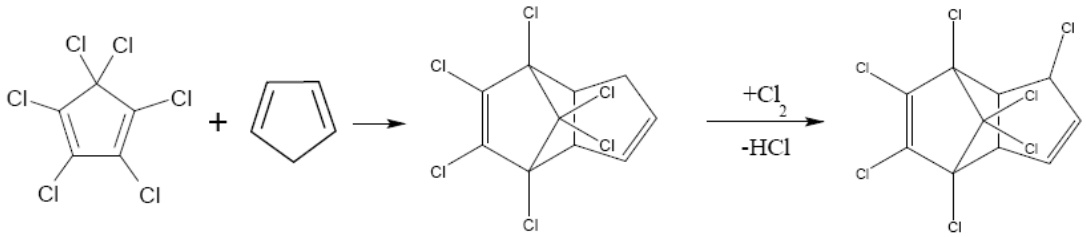



Figure 1. Synthesis of heptachlor.

The best yield is obtained by chlorination of chlordene with sulfonyl chloride ($SO_2Cl_2$) in the presence of benzoyl peroxide. Depending on the chlorination process the composition of the technical mixtures may differ.

Technical heptachlor usually contains about 72 % heptachlor, and 28 % related compounds including about 18 % trans-chlordane, 2 % cis-chlordane, 2 % nonachlor, 1 % chlordene, 0.2 % hexachlorobutadiene and 10 - 15 other compounds (WHO-IPCS, 2006). There are two enantiomeric forms of heptachlor. Technical heptachlor and technical chlordane contain racemic mixtures of heptachlor and the two enantiomers differ slightly in insecticidal activity while the racemate is most active (Miyazaki *et al.,* 1980).

In Figure 2, the structure of chlordane is shown.

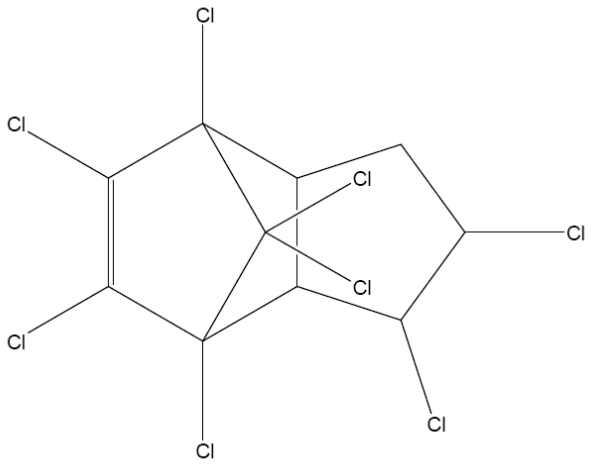



Figure 2. Structure of chlordane.

Heptachlor is rapidly converted by biotic and abiotic oxidation processes to the more stable and persistent heptachlor epoxide (2,3-epoxy-1,4,5,6,7,8,8a-heptachloro-3a,4,7,7a-hexahydro-4,7-methanoindane; CAS No. 1024-57-3; $C_{10}H_5Cl_7O$), see Figure 2. Heptachlor epoxide, also chiral, shows somewhat stronger insecticidal activity than the parent compound (Kirk-Othmer, 1981). The abiotic process results in a racemic mixture of heptachlor epoxide while the biotic processes result in predominantly the (+)-enantiomer (Müller and Buser, 1994; Müller *et al.,* 1997; Pfaffenberger *et al.,* 1994). Heptachlor epoxide may occur as both cis (or exo) and trans (or endo) isomers, with the epoxide-bridge on the same or opposite side of the dichloromethylene group, respectively. Studies have shown that the cis-isomer is the main product of both biotic and abiotic oxidations (Müller *et al.*, 1997) while small amounts of the trans (endo) isomer may also be formed in the abiotic oxidation (Buser and Müller, 1993).

Furthermore, irradiation of heptachlor by long wavelength UV-light converts it to a caged and chiral photoisomer by intramolecular cycloaddition, *i.e.* photoheptachlor, which is more insecticidal than heptachlor and heptachlor epoxide, and is especially more toxic to fish as compared with heptachlor (Buser and Müller, 1993; Zhu *et al.,* 1995; Podowski *et al.,* 1979), see Figure 2. Photoheptachlor is more persistent than heptachlor epoxide but with similar bimagnification potential (Zhu *et al.,* 1995; Strandberg *et al.,* 1998).

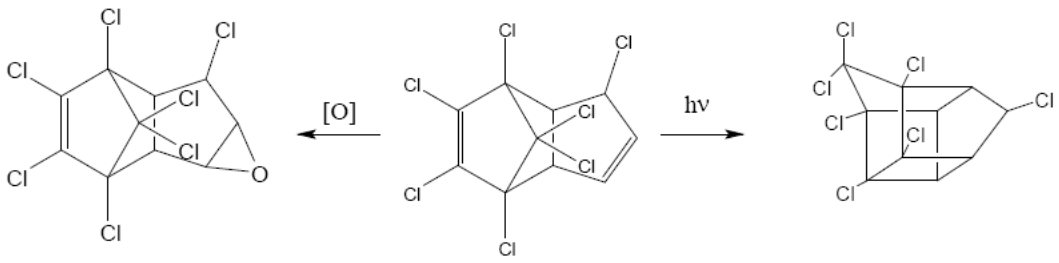
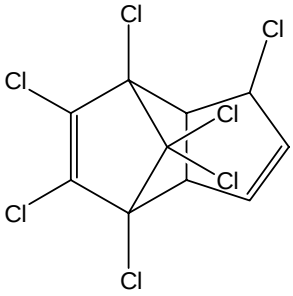
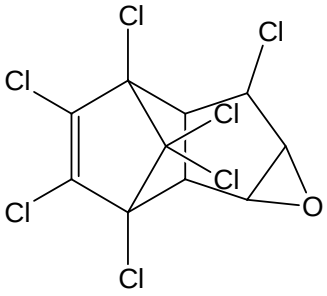



Figure 2. Conversion of heptachlor by epoxidation to heptachlor epoxide (left) and by photolysis to photoheptachlor (right).

The physical properties of heptachlor and heptachlor epoxide are summarised in Table 1. The differences in the reported constants are due to difficulties in measurements of partition properties of sparingly soluble and semivolatile substances. Salinity enhances the Henry's law constants (Cetin *et al.* 2006) as well as partition into organic solvents (salting-out). The vapour pressure and Henry's Law constants are unusually high for heptachlor in comparison with other organochlorine pesticides, and hence more prone to volatilisation as well as long-range air transport.

Table 1. Physical properties of heptachlor and heptachlor epoxide.

| **Structure and name** | Properties |
|---|---|
| **Heptachlor**<br>  | Synonyms: 3-chlorochlordene; 1,4,5,6,7,8,8a-heptachloro-3a,4,7,7a-tetrahydro-4,7-methano-1*H*-indene.<br><br>Molecular mass: 373.32<br><br>Melting point (°C): 93[a], 98[b]<br><br>Solubility in water (mg/L): 0.10 (15°C)[c], 0.18 (25°C)[c]<br><br>Vapour pressure, Pa (25°C): $4.0 \cdot 10^{-2}$ [d], $5.3 \cdot 10^{-2}$ [e], $3.1 \cdot 10^{-2}$ [f]<br><br>Log Kow: 6.1k, 4.4-5.5[g], 5.94[e]<br><br>Log Koc (mL/g): 4.9[h]<br><br>Henry's law constant, Pa·m$^3$/mol (25°C): 233[i], 29.75[j], 29.73[k], 62[l], 38[m] |
| **Heptachlor epoxide**<br>  | Synonyms: Epoxyheptachlor; 2,3-epoxy-1,4,5,6,7,8,8a-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindane.<br><br>Molecular mass: 389.32<br><br>Melting point (°C): 161.7[a], 166[b]<br><br>Solubility in water, mg/L: 0.11 (15°C)[c], 0.20 (25°C)[c], 0.350 (25°C)[n], 0.275 (25°C)[o]<br><br>Vapour pressure, Pa (20°C): $3.47 \cdot 10^{-4}$ [d,i]<br><br>Log Kow (mL/g): 5.1(calculated)[p], 3.65 and 5.40[i], 5.42[e]<br><br>Log Koc : 4.32[h]<br><br>Henry's law constant, Pa·m$^3$/mol (25°C): 3.2[i], 2.13[k], 2.3[l], 1.7[m] |

a) Plato, 1972; b) Ksiazczak and Nagata, 1995; c) Biggar and Riggs, 1974; d) Verschueren, 1996; e) Tomlin, 1997; f) Hinckley *et al.,* 1990; g) Simpson *et al.,* 1995; h) Ding and Wu, 1995; i) Montgomery, 1993; j) Thomas, 1990; k) Altschuh *et al.,* 1999; l) Cetin *et al.,* 2006; m) Shen and Wania, 2005; n) Hayes, 1982; o) US-EPA, 1987; p) Meador *et al.,* 1997.

The technical heptachlor is a soft white wax melting at 46 - 74°C and sparingly soluble in water, 0.056 mg/L (Kirk-Othmer, 1981). The pure compound is a white or light tan, crystalline solid at room temperature with a mild camphor- or cedar-like odour. Both heptachlor and heptachlor epoxide are very soluble in most organic solvents.

## 1.2. Production, use and environmental fate

### 1.2.1 Production and use

Production of heptachlor in the USA in 1971 was estimated to be 2700 tonnes. In addition, heptachlor was also produced as a component of technical chlordane. In 1970, most of the produced heptachlor was used in Europe (60 % as compared to: Asia 15 %, South America 15 %, Canada and the USA 5 %, Africa 5 %) (WHO-IPCS, 1988). Fendick *et al.* (1990) reports the agricultural consumption of heptachlor in the early 1970s in the USA to about 550 tonnes per year. Based on these figures the use in Europe in the early 1970s could have been more than 6000 tonnes per year.

According to a survey on sources of persistent organic pollutants, performed by the United Nations Environment Programme, the global import figures of heptachlor in 1993 and 1994, based on the response of 61 countries, were 389 and 435 tonnes, respectively, with South America and Oceania being the largest consumers (UNEP, 1996).

Heptachlor is a non-systemic stomach and contact insecticide with some fumigant action. It has, together with its epoxide metabolite, been shown to block gamma-aminobutyric acid (GABA) receptors in cockroach, *Periplaneta americana,* and other insects. The block appears to be non-competitive and no significant difference between the potencies of heptachlor and heptachlor epoxide has been demonstrated with respect to insect GABA receptors. It is non-phytotoxic at insecticidal concentrations (Worthing and Walker, 1987). Heptachlor was also used non-agriculturally to control termites and household insects (US-EPA, 1986; Worthing and Walker, 1987). Heptachlor was used extensively from 1953 to 1974 as a soil and seed treatment to protect corn, small grains, and sorghum from pests. It was used to control ants, cutworms, maggots, termites, thrips, weevils, and wireworms in both cultivated and uncultivated soils.

Most applications of this insecticide were banned or at least severely restricted in the early 1980s in many countries. Within the EU, marketing and use of heptachlor has been prohibited since 1984.

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 heptachlor.

In the US, nearly all registered uses of heptachlor were cancelled in 1974. There were exceptions for treatment of field corn, seed (for corn, wheat, oats, barley, rye, and sorghum), citrus, pineapple, and narcissus bulbs, which were phased out gradually over a 5-year period ending on July 1, 1983 (US-EPA, 1986). By April 1988, heptachlor could no longer be used

for the underground control of termites but the treatment of fire ants in power transformers remained until 2000. The registration of heptachlor in Canada was discontinued in 1985.

According to UNEP Chemicals (UNEP, 2003) summarising legal status for a number of persistent organic pollutants in 110 countries, heptachlor was still allowed in 20 countries including Albania and Belarus. 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 heptachlor also report that they have not found the compound within the country.

### 1.2.2. Environmental fate

Heptachlor partitions to the atmosphere from surface water and volatilization is an important mechanism of transport of heptachlor from land surfaces. When applied to orchard grass, approximately 90 % was lost in 7 days and similar results were obtained when applied to soil (Jury *et al.,* 1987). However, the major transformation product of heptachlor, heptachlor epoxide is much less volatile.

Heptachlor and heptachlor epoxide may both undergo direct photolysis in sunlight and are susceptible to photocatalysed reactions (Graham *et al.*, 1973; Ivie *et al.*, 1972; Podowski *et al.*, 1979). A major photolysis product of heptachlor is photoheptachlor which is quite stable with a potential for biomagnification (see structure in Figure 2). The presence of heptachlor epoxide in ambient air samples is due to the emission of this stable metabolite from soil where it is formed from heptachlor by microbial activity rather than to the photo-oxidation of heptachlor in the atmosphere (Bidleman *et al.,* 1998a,b).

In surface water, heptachlor is converted to 1-hydroxychlordene (see structure in 6.3) and heptachlor epoxide. After addition to river water at room temperature under sunlight exposure, a half-life for heptachlor of 3.5 days could be calculated. After four weeks approximately 60 % of the converted heptachlor remained as 1-hydroxychlordene and 40 % was converted to the epoxide. Heptachlor epoxide added to river water (pH 7.3 – 8) or to distilled water showed a half-life of at least 4 years (Eichelberger and Lichtenberg, 1971).

**Bioaccumulation and bioconcentration**

In shellfish and fish, heptachlor and heptachlor epoxide both show a high potential for bioaccumulation with bioconcentration factors (BCFs) between 1000 and 17,000 (Hawker and Connell, 1986; Geyer *et al.*, 1982; Hartley and Johnston, 1983; Lu and Wang, 2002; WHO-IPCS, 2006). In mammals and birds, the parent compound is rapidly converted to heptachlor epoxide, thus the biomagnification of heptachlor itself is not significant. On the other hand, biomagnification of the more persistent heptachlor epoxide is significant in both the aquatic and terrestrial food chains.

**Levels in the environment**

Ambient air concentrations of total heptachlor are generally in the pg/m$^3$ range. Higher values are expected to be found in agricultural areas where heptachlor was previously used as a pesticide and where the pesticide is still being released from the soil. Several monitoring studies have shown evidence of recent heptachlor usage, including, for example, elevated levels of heptachlor in air in Africa and the USA (Jantunen *et al.,* 2000; Karlsson *et al.*, 2000).

Heptachlor epoxide was detected in rain samples at concentrations ranging from 0.03 to 1 ng/L (Canada 1984) (Strachan, 1988), 0.1 to 1.5 ng/L (Poland) (Grynkiewicz *et al.,* 2001; Polkowska *et al.,* 2002), up to 7 (heptachlor up to 3) ng/L in typical agricultural locations (the Netherlands) (Hamers *et al.,* 2003). Heptachlor epoxide has also been detected in wastewater (82 – 1100 ng/L; median 200 ng/L) at a municipal wastewater treatment plant in Greece (Katsoyiannis and Samara, 2004).

Fish from several areas have been found to contain total heptachlor concentrations below 1 μg/kg wet weight or in the low μg/kg lipid weight range. Much higher values (in the 100 – 1000 μg/kg range) have however been reported from a number of areas such as the Ganges Estuary, Bangladesh, the Bay of Bengal, and the Göksu Delta (Turkey) (Ayas *et al.,* 1997; Jabber *et al.,* 2001; Das *et al.,* 2002). From the latter area also aquatic birds such as mallard (*Anas platyrhynchos*) and little egret (*Egretta garzetta*) were analysed showing total heptachlor levels at around 2700 μg/kg adipose tissue in mallards and 980 μg/kg in eggs of little egret.

Total heptachlor has also been found in toothed whale such as Northwest Atlantic pilot whales (*Globicephala melas*) with a mean concentration of total heptachlor between 39 and 56 μg/kg lipid (Weisbrod *et al*., 2000a) as well as in right whales (*Eubalaena glacialis*) where mean values as high as 1300 μg/kg lipid have been reported (Weisbrod *et al.*, 2000b).

## 1.3. Toxicology in laboratory animals and hazard characterisation for humans

Heptachlor has been evaluated several times by various international bodies within WHO (WHO-IPCS, 1984; FAO/WHO 1992; IARC, 2001), updates have been performed in WHO (WHO-IPCS, 2006) and by the Agency for Toxic Substances and Disease Registry (ATSDR, 2005). Most toxicological studies, particularly the early ones, have been carried out with technical heptachlor, which contains about 72 % heptachlor, and 28 % related compounds including about 18 % trans-chlordane, 2 % cis-chlordane, 2 % nonachlor, 1 % chlordene, 0.2 % hexachlorobutadiene and 10 - 15 other compounds (WHO-IPCS, 2006).

In the rat and mouse, acute oral $LD_{50}$s for heptachlor are 40 – 162 and 68 – 90 mg/kg b.w., respectively. The dose response curve for mortality is quite steep. The main acute target is the central nervous system showing hyper excitability, tremors, convulsions, and paralysis. Liver toxicity has also been reported. The acute toxicity of heptachlor epoxide and photoheptachlor is about four and 20 times, respectively, greater than that of heptachlor, whereas other metabolites or impurities (chlordane, 3-chlorochlordene, 1-hydroxychlordene and chlordane epoxide and nonachlor) are much less toxic (WHO-IPCS, 2006; Zhu, 1995).

Upon repeated exposure to heptachlor in mice (30 days) and rats (14 days) liver enlargement and histopathological signs of enlargement of centrilobular- and midzonal hepatocytes were seen. Such effects were seen at doses of 1.3 and 2 mg/kg b.w. per day and above this dose in mice and rats, respectively.

Clinical effects and effects on the liver have been observed in experiments in dogs, see chapter 5.9.

### 1.3.1. Long-term studies of carcinogenicity

Osborne-Mendel rats and B6C3F1 mice were given technical heptachlor in the diet for 80 weeks at two different dose levels which were changed during the study due to observable toxicity (NCI, 1977). The time-weighted average doses were 39 and 78 mg/kg diet and 26 and 51 mg/kg diet for male and female rats, respectively; and 6 and 14 mg/kg diet and 9 and 18 mg/kg diet for male and female mice, respectively. The average body weights of the rats at the highest doses were reduced compared with untreated controls. A dose-related mortality was observed for female rats, but not for the males. No increased tumour incidence was observed in the treated rats. A dose-related mortality was observed for the female mice. An increase in the combined incidence of hepatocellular carcinoma and "nodular changes" in male and female mice was considered significant by a review panel of the US National Academy of Sciences (US-NAS, 1977).

Three groups of male and female C3H mice were fed 0 and 10 mg heptachlor or 10 mg heptachlor epoxide per kg diet for 24 months. Liver histopathology was reviewed by the US National Academy of Sciences panel (US-NAS, 1977). Significant increases in the incidence of hepatocellular carcinomas in females but not in males given heptachlor and in both sexes for heptachlor epoxide were observed.

In a third study in mice (CD-1) on combined exposure to heptachlor and its epoxide a significant e.g. increased incidence of hepatocellular carcinomas and nodules was found in the groups at the highest dietary concentration, 10 mg (25 % heptachlor and 75 % heptachlor epoxide per kg diet) (Epstein, 1976; US-NAS, 1977).

In an initiation–promotion assay, heptachlor was active as a promoter after initiation by N-nitrosodiethylamine (Williams and Numoto, 1984).

### 1.3.2. Genotoxicity

Genotoxicity tests for heptachlor and its epoxide were reviewed by IARC (2001). A number of tests with heptachlor in bacteria, yeast, *Drosophila* and mammalian cells were negative. One exception was gene mutations at the *Tk* locus but not at the *Hprt* locus in rodent cells. An unpublished study by NTP cited in IARC (2001) showed a weak positive effect of heptachlor on sister chromatid exchange but not on chromosomal aberrations. There are fewer studies on heptachlor epoxide and these are negative. Hence it can be concluded that heptachlor and its epoxide show mostly negative responses in genotoxicity testing.

### 1.3.3. Intercellular communication and other biochemical effects

Heptachlor and its epoxide cause *in vitro* inhibition of gap junctional intercellular communication. Heptachlor is an inducer of CYP enzymes, NADPH reductase and glutathione-S-transferases in the liver (WHO-IPCS, 2006). Photoheptachlor is a much stronger inducer of cytochrome P450 (CYP) enzymes than heptachlor (Khan *et al.,* 1998). Heptachlor also trigger proliferation of quiescent hepatocytes and activated protein kinase C MAPKs *in vitro*. In mice, heptachlor interacted with protein kinase C, particularly the epsilon isoform, which is believed to be important for tumour promotion (Hansen and Matsumura, 2001). In human lymphocytes heptachlor down regulated tumour suppressor gene p53 and the retinoblastoma (Rb) gene. A number of pathways important for cell cycle regulation as well as apoptosis and growth factor receptor pathways can be affected by heptachlor in cells *in vitro* (WHO-IPCS, 2006).

### 1.3.4. Reproductive and developmental toxicity

Reproductive and developmental toxicity studies were recently reviewed by WHO (WHO-IPCS, 2006).

Fertility studies in rats injected with heptachlor subcutaneously resulted in LOAELs of 5 mg/kg b.w. per day for suppression of sex hormone levels, disruptions in female cyclicity, and delays in mating behaviour.

In developmental toxicity studies in rats and beagle dogs (see also chapter 5.9.), there were usually no clinical signs of maternal toxicity (dose-related alterations in weight gain) until mortality occurred (NOAEL for maternal toxicity = 3 mg/kg b.w. per day). In one study, reduced litter sizes were noted, but postnatal mortality of the pups was the most obvious finding (NOAEL for pre- or postnatal survival of pups = 6 mg/kg b.w. per day). Studies evaluating effects on reproductive organs did not note such effects. No teratological effects were observed (see also 1.3.5 Neurotoxicity). Recent developmental toxicity studies in rats showed both neurotoxicity and immunotoxicity with NOAELs and LOAELs, respectively, of 0.030 mg/kg b.w. per day (WHO-IPCS, 2006).

For studies in mink see chapter 5.8.

### 1.3.5. Neurotoxicity

There is accumulating evidence that the nervous system and its development are influenced by cyclodiene pesticides. Heptachlor and the more potent heptachlor epoxide appear to act on the $GABA_A$ receptor by binding to the chloride channel thereby blocking the inhibitory actions of the GABA neurotransmitter. The profile of effects produced by repeated heptachlor administration to female rats consisted of altered activity, hyperexcitability, and autonomic

effects (NOAEL = 2 mg/kg b.w. per day). Neurotoxicological studies on perinatal heptachlor exposure in the rat (0.03, 0.3, or 3 mg/kg b.w. per day) suggested developmental delays, alterations in GABAergic neuro-transmission, and neuro-behavioural changes, including cognitive deficits at all doses. It also appears that the dopaminergic system is particularly sensitive to heptachlor, which increases dopamine uptake, attributed to dopamine transporter induction (WHO-IPCS, 2006).

### 1.3.6. Immunotoxicity

Immunological studies in rats indicate the suppression of the primary IgM and secondary IgG anti-sheep red blood cell responses following perinatal exposure to all tested doses (0.03, 0.3, or 3 mg/kg b.w. per day) of heptachlor. Studies on peripheral mononuclear blood cells from monkeys showed immunomodulatory effects of heptachlor (WHO-IPCS, 2006).

### 1.3.7. Observations in humans

Available epidemiological studies on cancer mortality do not show a clear relationship to human heptachlor body burden. In several studies heptachlor epoxide concentrations in breast adipose tissue from women with mammary carcinomas were compared with samples from women with benign disease. In some of these studies a possible correlation between serum concentrations of heptachlor or its epoxide and breast cancer were observed. However, other pesticides were also present and in some cases correlated better with the tumour incidence. Hence, no definitive conclusion could be drawn (WHO-IPCS, 2006).

Other endpoints such as reproductive toxicity and neurological effects have been addressed in epidemiological studies, however in neither case was a clear relationship to heptachlor exposure shown (WHO-IPCS, 2006).

### 1.3.8. Evaluations and classifications

IARC (2001) evaluated the carcinogenicity of chlordane and heptachlor in 2000 and concluded that there is inadequate evidence in humans and sufficient evidence in experimental animals for the carcinogenicity of heptachlor; the overall evaluation was that heptachlor is possibly carcinogenic to humans (Group 2B).

Heptachlor was evaluated by WHO in 2006 (WHO-IPCS, 2006). The Committee noted that there were inadequate data in humans and the evaluation was therefore based on animal data. Heptachlor was considered to be carcinogenic in mice but not in rats and to act as a tumour promoter. Non-tumourigenic effects occurred at doses about $1/20^{th}$ of the tumourigenic doses.

A tolerable daily (TDI) intake of 0.0001 mg/kg b.w. was derived based on histopathological changes in the liver in dog studies with a NOAEL of 0.025 mg/kg b.w. per day. An

uncertainty factor of 200 was used (10 for intra- and 10 for inter species variation and an extra factor of 2 for inadequacy of the data base; WHO-IPCS, 2006).

## 2. Methods of analysis

According to Article 11 of Regulation (EC) No 882/2004 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 heptachlor and heptachlor epoxide in animal feed. Multi-residue procedures for polychlorinated biphenyls (PCBs) and pesticides including heptachlor and cis(exo)-heptachlor epoxide using high-resolution gas chromatography with electron capture detection (HRGC/ECD) and high resolution gas chromatography with mass spectrometry (HRGC/MS) in animal feeding stuffs 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 limits of quantification (LOQ) for heptachlor and heptachlor epoxide in feed by applying HRGC/ECD and HRGC/MS are each given as 2.0 and 0.5 μg/kg, respectively. Photoheptachlor and endo-heptachlor epoxide can also be detected by this method with a similar LOQ.

A number of other well-proven, validated multi-residue methods are available for the quantitative determination of heptachlor and heptachlor epoxide in various environmental matrices, including food, feed and other biological specimens (Muir and Sverko, 2006). Depending on the type of feed material, whether it is of plant or animal origin, the extraction and 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 heptachlor and heptachlor epoxide can be removed by gel permeation chromatography (GPC) and by adsorption chromatography on various solid phase materials (SPE), such as Florisil or alumina.

Clean up procedures that involve sulfuric acid need to be avoided when analysing for heptachlor and heptachlor epoxide since acid treatment of samples may degrade these compounds (Andersen *et al.,* 2001).

Due to the high electro negativity caused by the seven chlorine atoms of heptachlor and heptachlor epoxide, HRGC/ECD is the analytical method most commonly used. An efficient separation of heptachlor and heptachlor epoxide 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 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 since 1969 without noticeable general improvement in 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 the concentrations of the tested materials. Recent reports describing results obtained as inter-comparisons between laboratories show that analysis of heptachlor and heptachlor epoxide is still difficult for many laboratories (Carvalho *et al.,* 1999; Villeneuve *et al.*, 2004). For example, 10 and 14 out of 55 laboratories in a world wide inter-laboratory study analysing fish homogenate for heptachlor and heptachlor epoxide, respectively, provided acceptable results at median levels of 0.32 and 0.99 µg/kg dry weight (Villeneuve *et al.*, 2004). Similar outcome was obtained in a interlaboratory study for heptachlor and heptachlor epoxide in dried seaweed at median levels of 3 and 0.79 µg/kg dry weight (Carvalho *et al.,* 1999). In both studies, only indicative values could be assigned for heptachlor epoxide. Therefore, increased efforts for the improvement of analytical performance are highly recommended.

In the present analytical scheme both heptachlor and heptachlor epoxide are included. However, photoheptachlor has been found in fish and marine mammals at a level of one tenth to one third of the heptachlor epoxide. Photoheptachlor was also found in humans from Northern Quebec (Buser and Müller, 1993; Strandberg *et al.,* 1998; Zhu *et al.,* 1995). Therefore, the Panel suggests that photoheptachlor should be included in monitoring programmes on feed of marine origin.

## 3. Statutory limits

The use of heptachlor has been widely restricted since 1981 and has been banned since October 1984 in the EU by Council Directive 79/117/EEC of 21 December 1978[12] (see also Regulation 850/2004/EC[13]) which prohibited the placing on the market and use of plant protection products containing certain substances.

Heptachlor (sum of heptachlor and of heptachlor epoxide, expressed as heptachlor) is listed in the Annex to Directive 2002/32/EC. The maximum levels which apply to the sum of heptachlor and of heptachlor epoxide, expressed as heptachlor each pertain to a feedingstuff with a moisture content of 12 %. The maximum levels are for fats 0.2 mg/kg and for other feedingstuffs 0.01 mg/kg.

The Codex Alimentarius Commission adopted "extraneous maximum residue limits" (EMRL) in food commodities for heptachlor (defined as sum of heptachlor and heptachlor epoxide[14]). 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 recognized as acceptable in or on a food or agricultural commodity.

## 4. Occurrence in feed and animal exposure

Heptachlor 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.

[12] OJ L 33, 8.2.1979, p. 36
[13] OJ L 229, 29.6.2004, p. 5
[14] See EMRL values at http://www.codexalimentarius.net/mrls/pestdes/jsp/pest_q-e.jsp

As an insecticide, heptachlor is predominantly applied as a spray. Therefore, vegetables and crops with large and waxy leaf surfaces grown in areas with ongoing or recent use of heptachlor are more likely to contain elevated heptachlor and to a minor extent also heptachlor epoxide levels. In contrast, uptake of heptachlor by roots is generally low due to its low water solubility. Once taken up by animals, heptachlor is metabolised mainly to heptachlor epoxide (see chapter 6). Hence, different contamination patterns are to be anticipated in feedingstuffs of plant and animal origin.

In Belgium, a total of 870 single and compound feed samples were collected and analysed between 2000 and 2004. The analytical methods covered heptachlor and heptachlor epoxide at levels of detection between 2 and 10 µg/kg. All 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. Total heptachlor 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. The results for heptachlor are given as "sum of heptachlor and heptachlor epoxide". In 15 feed samples heptachlor could be determined at concentrations between 2 and 21 µg/kg. The highest level was found in animal fat, followed by citrus pulp (16 µg/kg) and miscellaneous feeds. In general, fat containing samples showed somewhat higher levels than specimens of plant origin.

In Finland, 14 feed samples of plant and animal origin were recently analysed for undesirable substances. In all samples heptachlor (expressed as sum of heptachlor and heptachlor epoxide) was below the limit of determination (LOD). This LOD was 10 µg/kg except for one sample of cod liver oil which had a limit of determination of 200 µg/kg, all based on 88 % dry matter.

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 total heptachlor above the limit of detection of 5 µg/kg.

Norway reported on the analyses of 33 feed samples for undesirable substances including heptachlor and heptachlor epoxide in 2004. The limit of determination was given as 2.5 µg/kg for heptachlor and 0.5 µg/kg for heptachlor epoxide. All samples were negative except for one fish meal sample that contained heptachlor epoxide at a concentration of 1.4 µg/kg. 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.

In 2003 and 2004, analyses for heptachlor, heptachlor epoxide and other undesirable substances in 15 fish meal samples were performed in the Czech Republic. None of the samples contained heptachlor and heptachlor epoxide above the LOD 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 heptachlor expressed as total heptachlor in fish

meal and fish oil ranged from 0.41 – 0.84 and 5.0 – 13.2 µg/kg, respectively (Icelandic Fisheries Laboratories, 2005).

The European Feed Manufacturers Federation (FEFAC) provided data on 37 fish feed, 22 fish oil and 21 fish meal samples. The FEFAC data show that the sum of heptachlor (heptachlor-exo-epoxide, heptachlor-endo-epoxide and heptachlor) in fish feed ranged from 0.3 - 10.1 µg/kg with an average of 4.0 µg/kg. Heptachlor-exo-epoxide was the predominant compound with a range of 0.2 - 7.7 (average: 2.9), followed by heptachlor-endo-epoxide with a range of 0.09 - 1.8 (average: 0.78) and heptachlor with a range of 0.01 - 1.4 (average: 0.32), each given as µg/kg. The sum of heptachlor levels in fish oil ranged from 1.3 - 15.8 with an average of 8.8 µg/kg. The major contribution to the sum of heptachlor could be attributed to heptachlor-exo-epoxide which on average amounted to more than 80 % of the sum (0.9-14.3, average: 7.0), followed by heptachlor-endo-epoxide (0.3 - 2.7, average: 1.2) and heptachlor (0.05 - 2.8, average: 0.61), each µg/kg. The sum of heptachlor levels in fish meal ranged from 0.15 – 6.3 µg/kg. The highest concentrations were found again for heptachlor-exo-epoxide (0.1 - 4.8, average: 1.5), followed by heptachlor-endo-epoxide (0.03 - 1.3, average: 0.4) and heptachlor (0.01 - 0.6, average: 0.13), each µg/kg.

Other data on 9 fish feed samples for salmonids also provided by FEFAC revealed three positive samples for heptachlor-exo-epoxide (1.3, 1.5 and 1.7 µg/kg). All other samples were negative at a limit of determination of <0.5 µg/kg.

In conclusion, the data on the occurrence of heptachlor and its metabolites 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 fish derived products. Heptachlor epoxide represents the predominant constituent in fish derived products, normally amounting to more than 80 % of the total heptachlor.

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

### 5.1. Introduction

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

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

Acute intoxication is expressed through stimulations 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 acute toxicity of the main metabolite heptachlor epoxide is about ten times greater than that of heptachlor.

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

### 5.2. Fish

Heptachlor shows variable acute toxicity to fish exposed via water. Reported 96 hour $LC_{50}$s of technical heptachlor range from 0.9 - 130 μg/L in various species. The $LC_{50}$s in rainbow trout (*Oncorhynchus mykiss*) were 7 - 43 μg/L (WHO-IPCS, 1984; Mayer and Ellersieck, 1986). Long-term exposure of fish to heptachlor via water may reduce survival in all life stages and induce a dose-related growth decrease. There are no data on dietary exposure of heptachlor in fish nor data on heptachlor epoxide and photoheptachlor exposure in fish via water or diet.

### 5.3. Ruminants

A single oral heptachlor treatment at 15 mg/kg b.w. in 1 - 2 week old calves showed no adverse clinical effects (Radeleff *et al*., 1955). Out of six calves treated with a single dose at 25 mg/kg b.w, two died, one was severely affected with neurological symptoms but recovered, and three did not show clinical symptoms. Five of seven calves treated with a single dose at 50 mg/kg b.w. died and the other two recovered after severe neurological symptoms.

Buck *et al.* (1959) treated orally single 1 - 2 week old calves with heptachlor at 2.5, 5 or 10 mg/kg b.w. per day. The calf at 2.5 mg/kg b.w. per day received 15 treatments before death and at 5 and 10 mg/kg b.w. per day, the calves died after 6 and 3 treatments, respectively. These authors also compared the effect of oral treatment with heptachlor epoxide to that of heptachlor in single 1 - 2 weeks old calves and found that the epoxide was approximately 10 times as toxic as heptachlor. Both compounds accumulated to produce intoxication when given in multiple doses. A dosage level of 0.2 mg/kg b.w. per day of heptachlor epoxide for 100 consecutive days produced no harmful effects. Dosage levels of the epoxide at 1 or 2.5 mg/kg b.w. per day resulted in death after 15 and 4 treatments, respectively.

Dairy cows were fed hay from a meadow treated with heptachlor at 1 or 4 ounces per acre, corresponding to approximately 0.06 and 0.22 mg/kg of heptachlor residue in the hay for 112 days (Harris *et al.,* 1956). The concentration of heptachlor in hay and the amount given resulted in an intake of approximately 0.002 and 0.007 mg/kg b.w per day. There was no detectable effect on milk production, feed consumption, or clinical health of the cows.

Histological examination of kidney, liver, muscle and fat tissue showed no abnormalities associated with the exposure.

Dairy cows were fed heptachlor at 50, 75, 100 or 200 mg/kg diet (one cow per dose) or heptachlor epoxide at 10 or 50 mg/kg diet (2 and 4 cows per dose, respectively) for 12 or 16 weeks (Link *et al*., 1964). There was no evidence of influence from the chemicals on the feed intake or milk production. The cows were followed for delayed toxic effects during a feed-off period which ranged from 6 weeks to 18 months. One cow fed 50 mg heptachlor epoxide/kg diet developed pyelonephritis at 11 weeks, but this was probably not treatment related. No (other) signs of intoxication were found. A NOAEL for heptachlor at 200 mg/kg diet (corresponding to approximately 6 mg/kg b.w. per day) and for heptachlor epoxide at 50 mg/kg diet (corresponding to approximately 1.5 mg/kg b.w. per day) may be derived from this study.

Technical grade heptachlor epoxide was fed to ten dairy cows (in groups of two) at dosage levels 0.2, 0.5, 1.5, 10 or 50 mg/kg daily ration for 12 weeks with the main purpose to study the relationship between the concentrations in the diet and in the body fat as well as the compound's persistence (Bruce *et al.,* 1965). A concentration of heptachlor epoxide of 50 mg/kg in the ration corresponded to approximately 1.8 mg/kg b.w per day. No reaction of the animals to the treatment was revealed but one of the cows at 50 mg/kg diet lost her calf a few days after birth. It was not known whether death was caused by the heptachlor epoxide treatment. The milk suckled by the calf contained heptachlor epoxide at about 20 mg/kg.

There are several reports on animals that had grazed on pastures treated with heptachlor. Most studies concern residues in the products (primarily heptachlor epoxide), and do not comment on possible toxic effects in the animals. Intoxications among cattle on pasture sprayed with heptachlor (up to 9 kg heptachlor per ha) have been reported (Dickson *et al.,* 1983; 1984). The first signs of neurological illness were seen one week later. Five cows and 3 calves of 50 cattle on the pasture died. The heptachlor epoxide level in the bone marrow from two of these cows was 180 and 230 and mg/kg fat. A similar level was found in the fat of the kidneys.

In adult sheep, no overt clinical effect was found after a single oral treatment of heptachlor at 25 mg/kg b.w. At a single dose of 50 - 100 mg/kg b.w. they died, recovered from severe intoxication or did not show overt clinical symptoms (Radeleff *et al*., 1955).

### 5.4. Horses

Intoxications among mares on pasture sprayed with heptachlor (up to 9 kg heptachlor per ha) have been reported (Dickson *et al.,* 1983; 1984). The first signs of neurological illness were seen one week later. Three of 18 horses on the pasture died. The heptachlor epoxide level in the bone marrow from two of these horses was 370 and 530 and mg/kg fat.

### 5.5. Pigs

Pigs were orally dosed with heptachlor at 0, (3 pigs) 2 (3 pigs) or 5 (2 pigs) mg/kg b.w. per day for up to 78 days (Dvorak and Halacka, 1975). Ultrastructural changes consisting of glycogen depletion and proliferation of agranular endoplasmatic reticulum, were observed in the liver of the lowest and highest dosed pigs after 78 and 27 days, respectively.

### 5.6. Birds

The reported oral $LD_{50}$ of heptachlor in male domestic chickens is 62 mg/kg b.w. (Sherman and Ross, 1961).

Heptachlor at 1 mg/kg diet (approximately 0.1 mg/kg b.w per day) was given to domestic chickens from the age of 3 weeks for up to 8 weeks (Giurgea *et al*., 1974; Giurgea and Manciulea, 1975). The treatment resulted in effects on plasma catecholamine content, liver glycogen content and adrenal ascorbic acid content indicating a state of stress response. Furthermore, the thymus weight was increased.

Technical grade heptachlor was fed to broiler chickens during the first 8 weeks of life at dietary levels up to 0.3 mg/kg (0.03-0.045 mg/kg b.w. per day) (Wagstaff *et al.,* 1977). No adverse clinical effects were observed.

In broiler chickens fed heptachlor in the ration at 0.1 or 0.3 mg/kg during the first 8 weeks of life, egg retention in the oviduct and increased frequency of heavier and deformed eggs were found at 25 to 29 weeks of age (Wagstaff *et al*., 1981). The concentrations correspond to 0.01 - 0.015 and 0.03 - 0.045 mg/kg b.w. per day, respectively.

When Japanese quail (*Coturnix japonica*) were fed heptachlor at 10 or 50 mg/kg diet from hatch, there was no obvious adverse effect on their growth or on reproductive success (Shellenberger and Newell, 1965). The dietary heptachlor concentrations correspond to approximately 0.6 and 3 mg/kg b.w. per day, respectively.

Egg-laying Japanese quail weighing between 123 and 278 g were given heptachlor at 0.05 to 1.0 mg/bird for 18 - 32 days to study effects on the embryos and chick viability (Grolleau and Froux, 1973). No effects were found on the bulk of eggs laid, on their mean weight, on the fecundity or on the embryo mortality. However, all dosage levels seemed to increase the death rate of chicks during the first 7 days compared with control chicks. No effects were revealed on sexual maturation or on the fecundity of the surviving progeny.

The use of heptachlor to control wireworms in wheat seeds was found to be responsible for reproductive effects and local population declines of wild birds such as American kestrels (*Falco sparverius*) and Canada geese (*Branta canadensis*) during the 1970s (Henny *et al.,* 1983; Blus *et al.*, 1984). Heptachlor epoxide concentration above 1.5 mg/kg in the kestrel eggs and above 10 mg/kg in the goose eggs were connected with reduced reproduction.

Heptachlor epoxide was fed to male and female domestic chickens at dietary levels of 0, 0.02, 0.1 or 0.2 mg/kg for 25 weeks (Wolvin *et al.,* 1969). A slightly higher mortality rate was

recorded for the group fed 0.2 mg/kg diet compared to controls. No effects on body weight gain, behaviour, or egg production were found. The hatchability was slightly decreased in eggs from the groups fed the two highest concentrations but the viability of the hatched chicks was not affected. Based on reduced hatchability a NOAEL of 0.02 mg/kg diet (0.002 mg/kg b.w. per day) was derived from the study.

### 5.7. Rabbits

The reported oral $LD_{50}$ of heptachlor in rabbit is 80 - 90 mg/kg b.w. (WHO-IPCS, 1984).

Rabbits that had been starved for 7 days were fed a diet containing heptachlor corresponding to approximately 2.5 mg/kg b.w. per day for 7 days (Shakoori and Haq, 1987a,b). The heptachlor containing diet induced aggravation of hepatic biochemical effects produced during the starvation in contrast to the effects in rabbits fed control diet where complete recovery from starving was observed. The same heptachlor diet was also given to rabbits for up to two weeks without prior starvation. Histological changes in the liver and some hepatic biochemical effects were revealed.

Twenty pregnant rabbits were orally treated with heptachlor epoxide at 5 mg/kg b.w. per day from day 6 to 11 of gestation (Wazeter *et al.,* 1969). Foetuses were recovered by Caesarean section on day 28. A significant increase in foetal weight was evident in the heptachlor epoxide treated group when compared with a corresponding unexposed control group. There were no teratogenic effects or other apparent compound-related effects on dams, foetuses or offspring.

### 5.8. Mink

Male mink (*Mustela vison*) were fed technical grade heptachlor in diet at 12.5, 25, 50 or 100 mg/kg diet for 28 days followed by a 7-day observation period (Aulerich *et al.,* 1990). The dietary concentrations correspond to 1.8, 3.0, 5.7 and 6.2 mg/kg b.w. per day, respectively. Diets that contained 25 mg/kg or more caused a significant decrease in feed consumption, while 50 mg/kg diet (or 3.0 mg/kg b.w.) or more reduced the body weights. Mortality (38 %) occurred only among those mink fed 100 mg/kg diet (or 6.2 mg/kg b.w.). Hyperexcitability and incoordination were observed prior to death. Mink fed 100 mg/kg diet had also reduced relative weight of the spleen and kidneys and increased relative weight of the adrenal glands. A NOAEL based on reduced feed consumption of 12.5 mg/kg diet corresponding to 1.8 mg/kg b.w. per day was established in this study.

Adult female mink were fed diets containing heptachlor at 6.25, 12.5 or 25 mg/kg diet prior to and throughout the reproductive period – altogether for 181 days (Crum *et al.,* 1993). The feed concentrations correspond to 1.0, 1.7 and 3.1 mg/kg b.w. per day, respectively. The purpose was to evaluate the effects on reproduction and offspring viability and to assess the extent of placental and mammary transfer of heptachlor to the offspring. Feeding 12.5 and 25 mg/kg reduced the feed consumption and the body weight of the female mink. The mortality

was 8, 67 and 100 % in the three dosage groups compared with 0 % in the control group. Clinical neurological signs were seen just prior to death. Whelping success rates were 83, 27 and 0 % in the three dosage groups compared with 67 % among the controls. Gestation length, litter size, birth weight and survival of kits were not significantly affected in the group given 6.25 mg/kg, but kits from females on the 12.5 mg/kg diet had lower weight and reduced survival rate. At three and six weeks of age, kit body weights in both 6.25 and 12.5 mg/kg groups were significantly less than those of controls. The LOAEL based on reduced kit growth was 6.25 mg/kg diet equivalent to 1.0 mg/kg b.w. per day.

### 5.9. Dogs

Two dogs given heptachlor in corn oil orally at 5 mg/kg b.w. per day died within 21 days, whereas three of four dogs given heptachlor at 1 mg/kg b.w. died within 424 days (Lehman, 1952).

Three dogs given heptachlor epoxide orally in dosages of 2, 4 and 8 mg/kg b.w. per day for 5 days per week died after 22, 10 and 3 weeks, respectively. Daily oral doses of 0.25 and 0.5 mg/kg b.w. did not cause clinical effects during 52 weeks, but 0.25 mg/kg b.w., estimated to be 6 mg/kg in the diet, was reported as the minimal dose producing pathological effects (unpublished report cited in FAO/WHO, 1992).

Diets containing heptachlor epoxide at 0.5, 2.5, 5 or 7.5 mg/kg were given to groups of 5 beagle dogs (3 females, 2 males, 23 - 27 weeks of age) for 60 weeks (Witherup *et al.,* 1958). No death attributed to the compound occurred, but the weight gain of the males showed inverse proportion to its concentration in diet. The liver weight was increased at 5 mg/kg diet and above. Degenerative liver changes were seen in one dog at the highest dosage level. The NOAEL was 2.5 mg/kg diet (corresponding to approximately 0.06 mg/kg b.w. per day) was identified.

Beagle dogs (four dogs per sex per dose) were fed heptachlor epoxide at concentrations of 0, 1, 3, 5, 7 and 10 mg/kg in the diet for two years (Wazeter *et al*., 1971a). Then, two dogs per sex per dose were sacrificed and examined *postmortem*, while the others were maintained on the control diet for an additional 6 months. Neither deaths nor compound-related behavioural changes were seen during the study. No significant effects of the treatment on body weights or food consumption were seen. The alkaline phosphatase activity was increased in males and females at 3 mg/kg and above. These increases tended to be more marked towards the end of the treatment period and to persist through the recovery period. The serum albumin and total protein levels were slightly decreased in 10 mg/kg diet male and female dogs during the treatment, and extended into the recovery period. After one year of treatment, the animals in the 7 mg/kg group also showed an increase in the alanine aminotransferase level, which also lasted into the recovery period. The liver weight increased in the 10 mg/kg male and female dogs relative to that of the controls, and the increase persisted with a slight attenuation during the recovery period. Histopathological examination of the dogs (two per sex per dose) sacrificed at the end of the treatment period showed an increase in the incidence of liver

changes (enlargement and vacuolisation of centrilobular hepatocytes) in animals at 3 mg/kg or above. These changes were also noted after 6 months of recovery. No compound-related histopathological changes were seen in the dogs receiving the 1 mg/kg diet. Based upon liver changes (increase in alkaline phosphatase) the NOAEL was 1 mg/kg diet (corresponding to 0.025 mg/kg b.w. per day).

Six- to 9-month-old beagle dogs (four per sex per dose) were fed heptachlor epoxide at dietary concentrations of 0, 1, 3, 5, 7, and 10 mg/kg for two years (Wazeter *et al.,* 1971b). When the females reached 14 months, they were mated with male dogs from the same dose group. The females were allowed to deliver and to nurse their pups. Four female and two male pups of the F1 generation were selected from each dose level to be the parental animals (P2) of the F2 generation. At age of 14 months, these animals were mated, and the pregnant females were allowed to deliver and to nurse their pups to 6 weeks of age, when the females and their pups were sacrificed. There was a significant increase in the mortality rate of F1 pups in the 10 mg/kg group. Only one male pup survived to scheduled sacrifice, and no female was available to serve as a P2 parental animal. There were slight increases in death rates of F2 pups at 3 and 7 mg/kg relative to the controls and the 5 mg/kg group had no pups. Based on mortality rate in offspring of later generations, the NOAEL was 1 mg/kg diet (corresponding to 0.025 mg/kg b.w. per day).

### 5.10 Cats

No information on toxicity to cats could be identified.

## 6. Toxicokinetics and tissue disposition

### 6.1. Absorption

Quantitative data on the absorption of heptachlor in laboratory animals, livestock and pets are lacking. However, several oral exposure studies conducted in rats (Radomski and Davidow, 1953; Tashiro and Matsumura, 1978), cattle (Harradine and McDougall, 1986; Petterson *et al.,* 1988) and broiler chickens (Wagstaff *et al.*, 1980) provide indirect evidence of absorption of heptachlor from the gastrointestinal tract, as indicated by the presence of this compound and/or related metabolites in serum, fat and other tissues.

### 6.2. Distribution

Heptachlor and heptachlor epoxide, which are highly lipophilic, concentrate in adipose tissue and other tissues with some fat content including the liver and the kidney. A single

intraperitoneal dose of $^{14}$C-heptachlor (1.64 mg/kg b.w.) administered to wether lambs resulted, 23 days after dosing, in residue levels (parent compound and metabolites) of about 1 mg/kg in kidney fat (Smith *et al.,* 1989). Concentrations were five fold lower in both liver and kidney tissue, and 6.3 fold lower in blood plasma, followed by even lower levels in spleen and muscle. The residues levels found in lactating ewes intraperitoneally dosed with $^{14}$C-heptachlor (2 mg/kg b.w.) were 9.5 mg/kg in mesenteric fat, 21 days after dosing (Holcombe *et al.,* 1988). Concentrations were 4.3 - 4.8 fold lower in liver and in whole blood and only traces (<0.1 mg/kg) were found in muscle.

Tissue distribution of photoheptachlor was studied in one adult male rabbit intraperitoneally injected with a single dose of $^{14}$C-photoheptachlor (0.8 mg/kg b.w.) (Feroz and Khan, 1979). Small amounts of residues (0.6 mg/kg b.w.) were detected in liver and fat of animals slaughtered 19 weeks post treatment, but most of the tissues did not contain radioactive material. In the same experiment, after a first intraperitoneal dose of $^{14}$C-photoheptachlor (0.8 mg/kg b.w.), two animals received 5 additional doses at the end of week 2, 4, 5, 9 and 11 and were slaughtered 7 or 13 weeks after the administration of the last dose. In both cases, tissue distribution ranked by concentration was as follows: adipose tissue > liver > kidney > brain.

Transplacental transfer of heptachlor was studied in adult female mink fed diets containing 0 (control), 6.25, 12.5, and 25 mg/kg technical grade heptachlor prior to and throughout the reproductive period (181 days). In the 6.25 and 12.5 groups, heptachlor epoxide concentrations in mink kits at birth was 0.9 and 3.1 mg/kg b.w., respectively, indicating a placental transfer of the chemical from the dams to the kits (Crum *et al.*, 1993).

Several authors have found heptachlor epoxide in human milk, blood plasma and adipose tissue (ATSDR, 2005). Adipose tissue samples from various body parts of people living in northeast Louisiana, an area of heavy agriculture, were taken during pathological examination. Heptachlor epoxide levels in the individual tissue samples ranged from 20 to 790 mg/kg (average = 239 mg/kg) for the study in 1980 and from 60 to 220 mg/kg (average = 159 mg/kg) from adipose tissue samples taken from other donors for the study in 1984 (Holt *et al.,* 1986).

The presence of heptachlor epoxide in the adipose tissue of stillborn infants (Wasserman *et al.,* 1974) and in the cord blood of newborns (D'Ercole *et al.,* 1976) demonstrates placental transfer of heptachlor and/or metabolites.

### 6.3. Metabolism

Heptachlor epoxide, the primary metabolite of heptachlor was first isolated and identified from adipose tissue of dogs which had been administered heptachlor at a dose level of 1 to 3 mg/kg b.w. for 12 - 18 months and of rats fed for 3 months a diet containing 30 mg heptachlor/kg feed (Davidow and Radomski, 1953; Radomski and Davidow, 1953). Afterwards, heptachlor epoxide was identified in pig (Halacka *et al.,* 1974), broiler chickens (Wagstaff *et al.,* 1980), cattle (Harradine and McDougall, 1986; Petterson *et al.,* 1988) and cow milk (Bache *et al.,* 1960). In addition, heptachlor epoxide has been detected in tissue

samples from 77 autopsies of humans performed from 1966 to 1968 in Hawaii at 1 to 32 µg/kg tissue, with highest concentrations in bone marrow and liver (Klemmer *et al.,* 1977). It has also been found in milk of lactating women (see chapter 8).

In analysing the faeces of rats given by gavage a single dose of $^{14}$C-heptachlor in corn oil corresponding to approximately 0.05 mg/kg b.w., Tashiro and Matsumura (1978) found 26.2 % heptachlor, 19.5 % 1-hydroxychlordene, 17.5 % 1-hydroxy-2,3epoxychlordene, 13.1 % heptachlor epoxide, 3.5 % 1,2-dihydroxychlordene, and 19 % unidentified compounds. An in vitro study using rat or human liver microsomes was performed by the same authors (Tashiro and Matsumura, 1978). In both cases, similar qualitative profiles were obtained. However, whereas in rat more than 95 % of heptachlor was biotransformed within 2 hours, mainly to heptachlor epoxide (this metabolite representing 85.8 % of total radioactivity), human microsomes, incubated in identical conditions with radio labelled heptachlor, produced only 31 % metabolites (20.4 % being heptachlor epoxide). These data suggest that heptachlor epoxide would be produced more efficiently in rat than in humans.

The metabolism of intraperitoneally administered $^{14}$C-photoheptachlor was studied in male rabbits by Feroz and Khan (1979). These authors found that radioactivity was excreted almost exclusively in urine. Analyses of urine revealed the presence of four metabolites, none of which corresponded to the parent compound. Because of the lack of reference standards, the characterisation of photoheptachlor metabolites was incomplete. Nevertheless the GC-MS analyses demonstrated an oxidative dechlorination of photoheptachlor and the elimination of this metabolite both in free and conjugated form. The nature of the conjugate was not elucidated.

Although glutathione conjugation is an important mechanism for the elimination of epoxides in mammals, no data were located demonstrating the occurrence of this metabolic pathway for heptachlor and heptachlor epoxide. However, the fact that 1) a treatment by *trans*-stilbene oxide (an inducer of epoxide hydrolases and glutathione-S-transferases) reduced the half-life of heptachlor by about 3 fold in rats (Rozman, 1984; Scheufler and Rozman, 1984), 2) heptachlor epoxide seems to be a good substrate *in vitro* for glutathione-S-transferases (Scheufler and Rozman, 1984) and 3) heptachlor epoxide is a potent inducer of hepatic glutathione transferase activities in mice (Moody *et al.,* 1991), all suggesting glutathione conjugation to be involved in the biotransformation of heptachlor epoxide *in vivo*.

Heptachlor is metabolized by the freshwater micro crustacean, *Daphnia magna*, to heptachlor epoxide or 1-hydroxychlordene. 1-Hydroxychlordene is then converted to 1-ketochlordene, 1-hydroxy-2,3-epoxy-chlordene, and their glucosides, sulphates, and other conjugates (Feroz *et al*., 1990).

Goldfish (*Carassius auratus*) intraperitoneally injected with $^{14}$C-heptachlor (38.2 µg/44g fish) were analysed 10 days after the treatment (Feroz and Khan, 1979). Five compounds were present in whole fish homogenates, of which 91.2 % was unchanged heptachlor, 5.4 % was heptachlor epoxide, 1.0 % was 1-hydrochlordene, 1.1 % was 1-hydroxy-2,3epoxychlordene and 1.2 % was an unidentified conjugate.

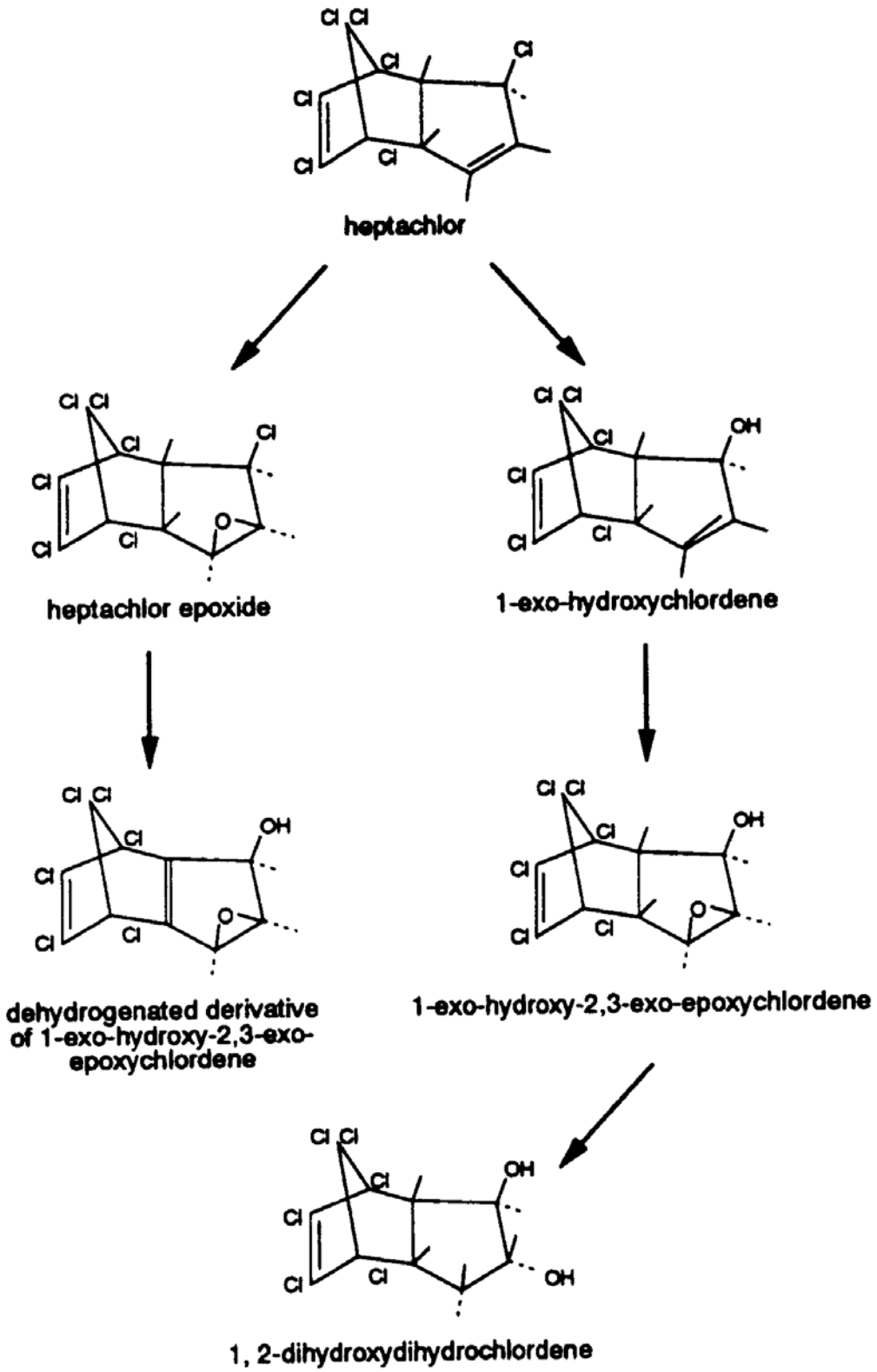



Figure 3. Suggested metabolic pathways of heptachlor in rats (adapted from Tashiro and Matsumura, 1978).

### 6.4 Excretion

Following a single oral gavage dose of $^{14}$C-heptachlor in male rats most of the radioactivity was eliminated in the faeces (Tashiro and Matsumura, 1978). One day after dosing, about 36 % of the dose had been eliminated in the faeces and only 1 % in urine. By day 10, approximately 62 % of the total radioactivity had been eliminated in the faeces, whereas 6 % was recovered from urine. Rozman (1984) reported that rats intraperitoneally dosed with 2 mg $^{14}$C-heptachlor/kg b.w. eliminated 45 to 55 % of the dose after 10 days in faeces, compared with 8 to 9 % in urine.

Smith *et al.* (1987) reported that lambs receiving a single intraperitoneal dose of $^{14}$C-heptachlor (1.64 mg/kg b.w.) excreted 34 % of the radioactivity over a 21 day period. Of this total, 66 % was in urine and 33 % was detected in faeces. Route and rate of heptachlor elimination therefore appear to be species dependent.

Heptachlor elimination half-life values are in the same order of magnitude in rats (5.1 days, Scheuffler and Rozman, 1984) and lactating ewes (11.7 days, Holcombe *et al.,* 1988). However, much higher values were reported for lactating dairy cows (6 to 8 weeks, Bruce *et al.,* 1965; Cook and Wilson, 1971) for pigs (10 to 12 weeks, Raisbeck *et al*., 1986) and for non lactating cattle (>20 weeks, Raisbeck *et al.,* 1986). Feroz and Khan (1979) reported a half-life value of about 10 weeks for photoheptachlor intraperitoneally administered to rabbits.

## 7. Carry-over and tissue concentration

### 7.1 Transfer into milk and eggs

Noble (1990) derived transfer ratios (concentration in milk or eggs relative to the concentration in the diet) from trials which have involved feeding heptachlor to dairy cattle and laying hens. Following heptachlor exposure, only heptachlor epoxide was detected in milk. The transfer ratio was within the range of 0.5 to 3.9 in dairy cattle. Following heptachlor epoxide exposure this ratio was between 3.8 and 6.1 according to Van den Hoek *et al.* (1975) and Vreman *et al.* (1980).

Kan and co-workers estimated that excretion of heptachlor epoxide via the eggs was 10 – 15 % of the heptachlor daily intake (Kan and Tuinstra, 1976; Kan and Jonker den Rooyen, 1978). The transfer ratios of heptachlor based on presence of heptachlor epoxide in eggs and calculated on a whole egg basis was between 0.5 and 2.9, as reviewed by Kan (1978) and by Noble *et al.*, 1990. Cummings *et al.* (1966) reported a ratio of 1.0 for heptachlor epoxide.

## 7.2 Tissue levels and bioaccumulation

Accumulation ratios (concentration in tissues relative to the concentration in the diet, usually calculated at the plateau level) for heptachlor and heptachlor epoxide in adipose tissue of different species have been reported.

Rats fed a diet containing heptachlor at concentrations of 0.1, 1, 5, 15 and 30 mg/kg feed for 12 weeks, showed an accumulation ratio in adipose tissue of about six for females and about one for males (Radomski and Davidow, 1953). Residues were identified as heptachlor epoxide, whereas no trace of parent compound was found.

Accumulation of heptachlor was investigated in fattening steers exposed for 58 weeks to heptachlor at a concentration of 0.19 mg/kg feed (Bovard *et al.*, 1971). At the end of the experiment, residue concentrations in fat were about five times greater than those in the feed. When heptachlor epoxide was fed to Shorthorn dairy cows at levels of 0.5, 1.5, 10 and 50 mg/kg feed (Bruce *et al.,* 1965), at the end of the 12 week exposure period, the residue concentrations in omental fat samples, taken by biopsy, were 7.1, 14.7, 83.5 and 293.4 mg/kg, corresponding to an accumulation ratio of 6 - 14.

In broiler chickens fed heptachlor at concentrations of 10, 30, 100 and 300 µg/kg feed for the first 8 weeks of life, residue concentrations in adipose tissue increased rapidly for one to two weeks and then tended to a plateau at concentrations about five times greater than those in the feed (Wagstaff *et al.*, 1980). This value is in agreement with those reported by Kan (1978) and Ritcey *et al.* (1972). In the study carried out by Wagstaff *et al.* (1980), it has been demonstrated that liver lipids and muscle lipids contained an average of 1.5 and 1.7 times higher concentrations respectively than did lipids extracted from adipose tissue. In addition, the ratio of the concentration of heptachlor with respect to its epoxide metabolite varied with tissue. Heptachlor accounted for about 5 % of the total residues in the liver at one week and this percentage decreased rapidly and reached a non-detectable value after ten weeks. In adipose tissue, the percentage of heptachlor in the total residues was about the same as in the liver at one week, but decreased more slowly. Muscle differed in that the percentage of heptachlor increased between the first and the second weeks to reach a maximum of about 20 % at two weeks. Thereafter, values in muscle decreased slowly and were at about 14 % at the end of the exposure period. An accumulation ratio of 3 to 5 can be calculated from this study. Higher values (approximately 13) were reported for heptachlor epoxide by Vos *et al.* (1972).

In female dogs (of unspecified weight and breed) treated daily by heptachlor administered *via* a gelatine capsule (1 mg/kg b.w.) for 26 weeks the concentrations in the fat at the end of the exposure period was approximately 500 mg heptachlor epoxide/kg adipose tissue (Radomski and Davidow, 1953).

Crum *et al*. (1993) investigated the accumulation of heptachlor epoxide in mink offspring when the dams were fed diets containing 6.25 mg/kg technical grade heptachlor prior to and throughout the reproductive period (181 days). A continued accumulation of heptachlor epoxide was observed in developing kits, and a mean whole-body concentration of 6.6 mg/kg

b.w. was detected at the end of the experiment (corresponding to a body burden of 4339 µg per animal).

Bioconcentration of heptachlor from water to fish is well documented and values in the range of 2000 – 17,000 have been reported for total heptachlor (Lu and Wang, 2002; WHO, 2006) but no data were found on the bioaccumulation occurring through dietary exposure.

## 8. Human dietary exposure

Prior to the ban, high human exposure to heptachlor occurred due to direct exposure as a result of its application. Currently the exposure of the general population is primarily to heptachlor epoxide via the diet especially from food of animal origin. Inhalation of vapours from contaminated soil and water, or direct contact with residual heptachlor from pesticide application is of minor importance for the general population.

Studies on recent dietary intake of total heptachlor are scarce.

Total Diet studies performed in the US between 1980 and 1998 showed that daily intakes of total heptachlor decreased from 19 ng/kg per day for infants, from 20 ng/kg per day for toddlers, and from 7 ng/kg per day for adults (Gunderson, 1988) to less than was <0.1 ng/kg body weight per day for all age/sex groups (WHO-IPCS, 2006).

Intake studies performed across Canada between 1993 and 1998 resulted in average dietary intakes for all ages (male and female) of 0.08 and 0.16 ng/kg b.w. per day for total heptachlor (Health Canada, 2004).

Estimations of the dietary intake of total heptachlor in Poland during the period 1970 - 1996 were less than 10 ng/kg b.w. (Falandysz, 2003). The main sources were thought to be meat, meat products, and animal fats (WHO-IPCS, 2006). More recent data were not available. In breast adipose tissue sampled in the period 1997 - 2001 the concentration was usually about at or below the limit of quantification 2.5 - 6 µg/kg of fat) (Struciński *et al.,* 2002).

In the Czech Republic, heptachlor epoxide were as determined in the period 2004/2005 determined in 220 composite food samples (consumption market baskets) representing 205 food types and in the form of 3696 individual samples. Samples with the highest values were: liver sausage, marinated fish, butter and cheese. The estimated daily dietary intake for adults was less than 4 ng/kg b.w. (Ruprich, 2006).

In Germany, heptachlor epoxide is routinely found in human milk. Since 1980 almost 40,000 human milk samples were analysed in Germany for organochlorine pesticides, including cis(exo)-heptachlor epoxide. While the mean levels in 1979 - 1981 still amounted to 33 µg/kg milk fat, they decreased to 7 µg/kg milk fat in 1990 and 6 µg/kg milk fat in 1997. In the last

10 years the levels of cis (exo)-heptachlor epoxide is in the range or lower than the analytical limit of detection (BGVV, 2001).

In the framework of the 3rd WHO human milk field study (Malisch *et al.,* 2004), heptachlor epoxide was 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 pools from six non-European countries (Brazil, Egypt, Fiji, Hong Kong, Philippines and USA). The heptachlor epoxide levels ranged from < 1 – 13 µg/kg milk fat with median and mean values of 2 and 3.1 µg/kg milk fat, respectively. If only the European countries are considered, the heptachlor epoxide levels range from 1.3 – 13 µg/kg milk fat with median and mean values of 3.3 and 4.2 µg/kg milk fat, respectively. While the lowest levels in the European specimens were found in the pools from Ukraine, Russia, and Czech Republic, the highest level was determined in two pools from Bulgaria and Luxembourg.

Assuming an average daily intake of 800 ml breast milk with a fat content of 3.5 % for an exclusively breast fed baby weighing five kg, a heptachlor epoxide concentration of 3.3 µg/kg milk fat (median value for European countries of 3rd WHO human milk field study) would result in a median daily intake of around 20 ng/kg b.w.

The available data indicate that the average daily intake of total heptachlor in the EU is well below the tolerable daily intake of 100 ng/kg b.w.

## CONCLUSIONS

*Production, use and environmental fate*

- Heptachlor was commercially introduced as a non-systemic contact insecticide in 1945. Heptachlor was also a major constituent (about 10 %) of technical chlordane. Technical heptachlor usually contains about 72 % heptachlor, and 28 % related compounds including about 18 % trans-chlordane, 2 % cis-chlordane, 2 % nonachlor, 1 % chlordene, 0.2 % hexachlorobutadiene and 10 - 15 other compounds.
- Heptachlor was used for agricultural purposes, soil and seed treatment and wood protection. Heptachlor is banned for use in the European Union since 1984 and in most other countries worldwide.
- In the environment heptachlor is rapidly converted to heptachlor epoxide and photoheptachlor. All are lipophilic and the latter two are persistent in the environment and tend to accumulate in the food chain; photoheptachlor is found in fish and marine mammals. The half-life of heptachlor-epoxide in the air soil and water has been estimated to be in the range of months to many years.

*General toxicological effects*

- Heptachlor shows moderate acute toxicity compared with other organochlorine pesticides. $LD_{50}$s are in the range of 40 – 162 mg/kg b.w. for rats and mice.
- Heptachlor epoxide and photoheptachlor are more toxic than heptachlor. The main target organs are the nervous system and the liver. Heptachlor also affect the reproductive and the immune system. Heptachlor and heptachlor epoxide cause liver tumours in mice but are not genotoxic. Heptachlor is classified by IARC as possibly carcinogenic to humans (group 2B). WHO has established a TDI of 0.0001 mg/kg b.w.

*Adverse effects of heptachlor in target animals*

- Heptachlor shows a range of toxicity to different fish species exposed via water ($LC_{50}$, 96 hours, 0.9 – 130 μg/L). No data has been found on heptachlor epoxide and photoheptachlor. No oral studies in fish have been identified.
- In ruminants, a NOAEL for heptachlor of 200 mg/kg diet (corresponding to 6 mg/kg b.w. per day) and for heptachlor epoxide of 50 mg/kg (corresponding to 1.5 mg/kg b.w. per day) diet was established for cattle. Calves seem to be more susceptible.
- In pigs, ultrastructural changes in the liver were observed at a dose of 2 mg heptachlor/kg b.w. per day.
- For heptachlor a LOAEL in domestic chicken based on egg production of 0.1 mg/kg diet (corresponding to 0.010 - 0.015 mg/kg b.w. per day) was found. For heptachlor epoxide a NOAEL based on hatchability in domestic hens of 0.02 mg/kg diet (corresponding to 0.002 mg/kg b.w. per day) was identified.
- In mink a LOAEL for heptachlor of 6.25 mg/kg diet (corresponding to 1.0 mg/kg b.w.) based on reduced kit growth was identified.
- In dogs for heptachlor epoxide a NOAEL of 1 mg/kg diet (corresponding to 0.025 mg/kg b.w.) based on liver toxicity was found. For heptachlor no NOAEL or LOAEL can be derived.
- No NOAEL or LOAEL for rabbits can be derived. No information on long term exposure in other relevant species such as sheep, goats, horses or cats was identified.

*Contamination of feed*

- In general, total heptachlor (heptachlor and heptachlor epoxide) is not frequently found in feed commodities. When present, it is mostly in fish derived products and only very infrequently in feed materials of plant origin. Heptachlor epoxide is the predominant contaminant. The concentrations found are in the low μg/kg range and thus well below those that have been found to cause adverse effects in animals.
- Elevated levels may occur in feed commodities that originate from areas where heptachlor has recently been or still is used.

*Fate in animals and carry over*

- Total heptachlor accumulates in lipid rich tissues mainly as heptachlor epoxide.
- The half-life of total heptachlor varies from several days in rats to more than 20 weeks in non-lactating cattle. No information was found for humans.
- Following heptachlor exposure, only heptachlor epoxide was detected in milk. The transfer ratio (concentration of heptachlor epoxide in milk fat relative to the concentration in the diet) was within 0.5 to 3.9 range in dairy cattle. Following heptachlor epoxide exposure this ratio was between 3.8 and 6.1.
- The transfer ratios of total heptachlor in whole eggs were between 0.5 and 2.9.
- The accumulation ratio of total heptachlor (concentration in tissues relative to the concentration in the diet) in steers, dairy cows and broiler chickens varied from 5 to 14. No information was identified for fish.

*Human exposure*

- Data from human milk monitoring programmes performed in various EU Member States as well as from the few available exposure studies, show a considerable decline of more than 90 % in human exposure to total heptachlor over the past three decades.
- Food of animal origin is the major source of human exposure to heptachlor epoxide. Available data indicate a recent dietary intake for adults and children in the sub ng/kg b.w. per day range which is two to three orders of magnitude below the tolerable daily intake of 0.0001 mg/kg b.w. (100 ng/kg b.w.).
- Recent median exposure of exclusively breastfed infants in the EU was estimated to be around 20 ng/kg b.w. per day.

## DATA NEEDS AND RECOMMENDATIONS

- Besides heptachlor and heptachlor epoxide, the analyses of feed samples, especially of marine origin, should also include the determination of photoheptachlor.
- In the clean-up of samples, treatment with sulfuric acid must be avoided in order to prevent the decomposition of the analytes.
- Inter-comparisons performed on biological samples revealed large discrepancies in the performance of laboratories, indicating scope for improvement of the analytical methods.
- Toxicity and kinetic studies in fish exposed to heptachlor and heptachlor epoxide via the diet are lacking and should be conducted.

- 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. 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.
- Special attention should be paid to the control of feed materials coming from areas of the world where heptachlor has recently been or still is used.

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