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

HEXACHLOROBENZENE

AS UNDESIRABLE SUBSTANCE IN ANIMAL FEED

Question N° EFSA-Q-2005-185

Adopted on 13 September 2006

SUMMARY

Hexachlorobenzene (HCB) was introduced as an agricultural pesticide in 1945, and was banned in 1981 for agricultural use in the European Community. Nevertheless, it is still used to some extent as an industrial chemical and is still released to the environment during incineration and, to some extent, as a by-product from the manufacture of industrial chemicals and several pesticide formulations. HCB is quite volatile, highly lipophilic and among the more persistent environmental pollutants. As a result, it can be transported over long distances and is bioaccumulated in fatty tissues. HCB is ubiquitous in the environment, and is present in environmental and biological samples world-wide. HCB is included in the Stockholm convention on persistent organic pollutants (POPs)[1] and the United Nations Economic Commission for Europe (UNECE) Convention on long-range transboundary air pollution protocol on POPs (CLRTAP-POP)[2].

In its evaluation of this contaminant, the CONTAM Panel examined occurrence data to assess the levels that are currently found in the environment and in food and feed. Fish derived products, particularly fish oils, were generally found to contain the highest levels of HCB. But high levels were also occasionally found in plant products such as pumpkin seeds as well as in vegetable oils from contaminated areas.

HCB is readily absorbed in humans and animals. It has low acute toxicity. The liver is the predominant organ to be affected resulting in enzyme induction and porphyria. HCB is immunotoxic and causes ovarian toxicity in monkeys at a very low dose. Mink and Japanese quail seem to be among the most susceptible animal species. HCB is classified by IARC as a possible human carcinogen based on tumourigenic effects observed in experimental animals. HCB in some tests exhibits weak mutagenic activity and therefore a genotoxic mode of action could not be completely excluded.

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

Despite its presence in the environment, and in many foodstuffs and animal feed, the data show a considerable decline of up to 90 % in human HCB exposure over the last twenty years. Recent dietary HCB intake for adults and children (breastfed infants excluded) ranges up to a few ng/kg body weight (b.w.) per day which is far below the suggested health based guidance value of 170 ng/kg b.w. per day. Furthermore, the margin between the dose causing liver tumours in rats and the human exposure range indicates low concern from a public health point of view.

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LIST OF ABBREVIATIONS AND ACRONYMS

ATSDR	Agency for Toxic Substances and Disease Registry
B.w.	Body weight
CAS	Chemical Abstract Service
CEN	European Committee for Standardization
CLRTAP	Convention on long-range transboundary air pollution
EMRL	Extraneous maximum residue limits
EPA	Environmental Protection Agency
FAO	Food and Agriculture Organization
GC	Gas chromatography
HCB	Hexachlorobenzene
HCH	Hexachlorocyclohexane
HR	High resolution
IARC	International Agency on Research on Cancer
IPCS	International Programme on Chemical Safety
LD_{50}	Dose that causes death among 50 % of treated animals
LOAEL	Lowest observed adverse effect level
LOEL	Lowest observed effect level
ML	Maximum level
MRL	Maximum residue level
MS	Mass spectrometry
NOAEL	No observed adverse effect level
PCP	Pentachlorophenol
POPs	Persistent organic pollutants
SCAN	Scientific Committee on Animal Nutrition
TDI	Tolerable daily intake
UNECE	United Nation Economic Commission for Europe
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[3] replaces since 1 August 2003 Council Directive 1999/29/EC of 22 April 1999 on the undesirable substances and products in animal nutrition[4].

The main modifications can be summarised as follows:

- extension of the scope of the Directive to include the possibility of establishing maximum limits for undesirable substances in feed additives;
- deletion of the existing possibility to dilute contaminated feed materials instead of decontamination or destruction (introduction of the principle of non-dilution);
- deletion of the possibility for derogation of the maximum limits for particular local reasons;
- introduction of the possibility for 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").

Introduction of the principle of non-dilution is a particularly important and far-reaching measure. In order to protect human and animal health, it is important that the overall contamination of the food and feed chain is reduced to a level that is as low as reasonably achievable providing a high level of human health and animal health protection. Deletion of the possibility of dilution is a powerful incentive and should encourage all operators throughout the food and feed chain to apply the necessary prevention measures to avoid contamination as much as possible. Prohibition of dilution accompanied by the necessary control measures will effectively contribute to safer feed.

During the discussions leading up to adoption of Directive 2002/32/EC, the Commission made a 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. Following through on that commitment, in March 2001, the Commission asked the Scientific Committee on Animal Nutrition (SCAN) to provide these updated scientific risk assessments to enable the Commission to finalise its review as soon as possible (Question 121 on undesirable substances in feed)[5].

[3] OJ L140, 30.5.2002, p. 10
[4] OJ L 115, 4.5.1999, p. 32
[5] Summary record of the 135th SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu/comm/food/fs/sc/scan/out61_en.pdf)

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

Nevertheless, it was 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

Hexachlorobenzene (HCB) was used in the past as an agricultural pesticide. As hexachlorobenzene breaks down very slowly, it can remain in the environment for a long time.

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

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

- Directive 76/895/EEC of 23 November 1976 relating to the fixing of maximum levels for pesticide residues in and on fruit and vegetables[8];
- Directive 86/362/EEC of 24 July 1986 on the fixing of maximum residue levels for pesticide residues in and on cereals9;
- Directive 86/363/EEC of 24 July 1986 on the fixing of maximum residue levels for pesticide residues in and on foodstuffs of animal origin[10];
- Directive 90/642/EEC of 27 November 1990 on the fixing of maximum residue levels for pesticide residues in and on certain products of plant origin, including fruits and vegetables[11].

Before 1997, MRLs were fixed only for raw commodities. Council Directive 1997/41/EC of 25 June 1997[12] amending the directives mentioned above, provided for a system applicable from 1 January 1999 setting MRLs for processed products and composite foodstuffs, based on the MRLs that had been 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

[6] Opinion of the Scientific Committee on Animal Nutrition on Undesirable Substances in Feed, adopted on 20 February 2003, updated on 25 April 2003 (http://europa.eu.int/comm/food/fs/sc/scan/out126_bis_en.pdf)
[7] OJ L 33, 8.2.1979, p. 36
[8] OJ L 340, 9.12.1976, p.26
[9] OJ L 221, 7.8.1986, p. 37
[10] OJ L 221, 7.8.1986, p. 43
[11] OJ L 350, 14.12.1990, p. 71
[12] OJ L 184, 12/07/1997, p. 33

commodity by application of an appropriate dilution or concentration factor and for composite foodstuffs MRLs are calculated taking into account the relative concentrations of the ingredients in the composite foodstuffs. Following on from the coming into force of Directive 1997/41/EC, since 1 January 1999, the pesticide residue legislation applies also to animal feedingstuffs.

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

However some problems have been observed in implementing the pesticide residue legislation to animal feedingstuffs and are listed below.

- Compound feed is composed of a relatively high number of ingredients, of which several are processed products (by-products). It is not immediately evident which MRL is applicable to such compound feed as it involves many calculations, 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 used for animal feed (no food use) such as pastures, roughages, forages, fish oil and fish meal.

HCB is listed in the Annex to Directive 2002/32/EC.

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

Directive 2002/32/EC	
ML for HCB 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 HCB applicable to the product as marketed	
Product	**mg/kg**
Fruit and vegetables	0.01
Oilseeds	0.02
Cereals	0.01
Meat (fat)	0.2
Milk	0.01
Eggs	0.02

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

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

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 HCB in animal feed.

This scientific opinion should comprise the:

- determination of the toxic exposure levels (daily exposure) of HCB 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 HCB from the feed to the products of animal origin results in unacceptable levels of HCB 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 HCB 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 HCB:
    - to the overall exposure of the different relevant animal species to HCB;
    - 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

Hexachlorobenzene (HCB) is a chlorinated aromatic hydrocarbon which has been used as both a pesticide and as an industrial chemical. As a fungicide it was first introduced in 1945 (Yersin *et al.*, 1945) for seed treatment, especially for control of bunt of wheat. While its intentional production has declined during the past two decades, HCB is still formed as a by-product during the manufacture of industrial chemicals and several pesticide formulations. Moreover, it has been detected in the flue gas and the fly ash of municipal incinerators and other thermal processes. HCB is quite volatile, lipophilic and very resistant to breakdown in the environment. As a result, it can be transported over long distances and is bioaccumulated in fatty tissues.

Although there is a complete ban in the European Union on the use of HCB as a pesticide since 1981, it is still produced as an industrial chemical in other regions and continuously generated in thermal processes. It is included in the Stockholm convention on persistent organic pollutants (POPs)[14] and the United Nations Economic Commission for Europe (UNECE) Convention on long-range transboundary air pollution protocol on POPs (CLRTAP-POP)[15]. HCB is ubiquitous in the environment, and has been measured in environmental and biological samples world-wide.

1.1. Synthesis and chemistry

Industrial synthesis of HCB is mostly achieved through chlorination of benzene using ferric chloride as a catalyst (Figure 1). It can also be synthesized by distillation of residues from the production of tetrachloroethylene or by refluxing hexachlorocyclohexane (HCH) isomers with sulfuryl chloride or chlorosulfonic acid in the presence of a ferric chloride or aluminium catalyst (WHO-IPCS, 1997).

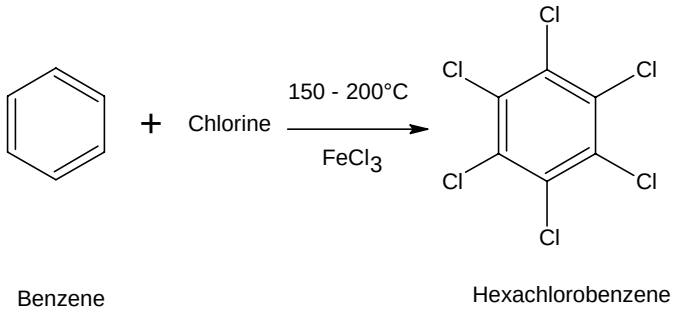

Figure 1. Synthesis of HCB through chlorination of benzene with ferric chloride.

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

Synonyms that have been used for HCB in the past include esachlorobenzene, perchlorobenzene, pentachlorophenyl chloride or phenyl perchloryl. Technical grade HCB contains about 98 % HCB, 1.8 % pentachlorobenzene and 0.2 % 1,2,4,5-tetrachlorobenzene (WHO-IPCS, 1997). Other impurities that were identified in technical grade HCB include octa- and heptachlorodibenzofurans, octachlorodibenzo- *p*-dioxin and decachlorobiphenyl (Villanueva *et al.,* 1974; Goldstein *et al.,* 1978). Octachlorodibenzo-p-dioxin and octachlorodibenzofuran concentrations up to 212 and 58 mg/kg technical HCB were reported respectively (LfU, 1995).

HCB (CAS registry number: 118-74-1) is a white crystalline powder with a melting point of 230°C. It sublimates at 322°C. The vapour pressure was determined as 2.3 x 10^{-3} Pa at 25°C (WHO-IPCS, 1997). Henry's Law Constants of 58.8 (ATSDR, 2002) and 131 Pa m^3/mol (WHO-IPCS, 1997; Eurochlor, 2005) have been reported, With a solubility of 5 - 6 µg/L HCB is virtually insoluble in water at 25°C but slightly to very soluble in most organic solvents. Log K_{OW} of 5.2 – 6.5 (LfU, 1995; WHO-IPCS, 1997; ATSDR, 2002) and Log K_{OC} values of 3.6 – 6.1 (ATSDR, 2002) were reported. HCB is very stable even to acids and alkaline.

1.2. Production, use and environmental fate

1.2.1. Production and use

The major agricultural application for HCB was as a seed dressing for crops such as wheat, barley, oats and rye to prevent the growth of fungi. It was marketed under trade names, such as Amatin, Anticarie, Bunt Cure, Bunt-No-More, Co-op Hexa, Granox NM, Julin's Carbon Chloride, No Bunt, No Bunt 40, No Bunt 80, No Bunt Liquid, Sanocide, Smut-Go, Snieciotox, HexaCB (WHO-IPCS, 1997). The use of HCB in such applications was discontinued in many countries in the 1970s due to concerns about adverse effects on the environment and human health.

There is no information that HCB might still be used as a pesticide in any country. The only listed notification of production and use of closed-system site-limited intermediates pursuant to note (iii) of annex A and note (iii) of annex B of the Stockholm Convention regarding HCB, originates from China. It includes 3000 - 4000 tonnes for the production of sodium pentachlorophenolate (Na-PCP).

In industry, HCB was used directly in the manufacture of pyrotechnics, tracer bullets and as a fluxing agent in the manufacture of aluminium. HCB has also been used as a wood-preserving agent, as a porosity-control agent in the manufacture of graphite anodes and as a peptizing agent in the production of rubber for tyres (WHO-IPCS, 1997).

There are few recent data on the quantities of the intentional production of HCB. Worldwide production of HCB from 1978 to 1981 has been estimated at 10,000 tonnes/year (Rippen and Frank, 1986). HCB was in 1978 produced/imported into the European Community at around 8000 tonnes/year (Rippen and Frank, 1986). One company in Spain produced some 150 tonnes of HCB annually (IARC, 1979) during the 1970s. Approximately 1500 tonnes of HCB

was manufactured annually in Germany for the production of a rubber auxiliary (BUA, 1994), but this production was discontinued in 1993. Except for the Chinese production of HCB as an intermediate mentioned above, it has not been possible to identify any information on current intentional production and use of HCB.

Despite restrictions imposed on production, HCB continues to be released into the environment from a number of sources. These are e.g. (incomplete) combustion, leakage from old dump sites and inappropriate manufacture and disposal of wastes from the manufacture of a number of chlorinated compounds, such as chlorinated solvents, aromatics and pesticides.

It has been estimated that > 5000 tonnes HCB/year were produced as a by-product resulting from tetrachloroethylene production in Germany in 1980 (Rippen and Frank, 1986). However, estimates for Europe (Johnson, P.G., European Chlorinated Solvent Association personal communication to IPCS in 1996), indicate that up to 4000 tonnes/year of HCB were produced as a by-product during certain tetrachloroethylene production processes and that over 99 % of this by-product was incinerated at high temperature. Jacoff *et al.* (1986) estimated that approximately 4130 tonnes of HCB was generated annually as a waste product in the USA and that nearly 77 % of this was produced during the manufacturing of three chlorinated solvents: carbon tetrachloride, trichloroethylene and tetrachloroethylene. The remainder was produced by the chlorinated pesticide industry. Although the concentrations of HCB in distillation bottoms (residues that form at the bottom of a distillation unit) were estimated to be 25 %, 15 % and 5 %, respectively, for tetrachloroethylene, carbon tetrachloride and trichloroethylene, analysis of the respective final products failed to detect HCB at a detection limit of 2 µg/L (WHO-IPCS, 1997).

HCB has also been identified as an impurity in pesticides such as pentachloronitrobenzene, dacthal, chlorothalonil, pentachlorophenol, atrazine, simazine, propazine and maleic hydrazide. Recently, the Food and Agriculture Organization of the United Nations established a maximum content of 0.04 g/kg for HCB as impurity in its specification for technical chlorothalonil and its related formulations (FAO, 2005).

Incineration is an important source today of HCB in the environment. Emission levels from incinerators are very site-specific, and therefore generic levels are difficult to estimate. Earlier information yielded a crude estimate of the total HCB released from all municipal incinerators in the USA to be 57 - 454 kg/year (US EPA, 1986), but levels currently emitted are not known. For Europe, estimates of total HCB emissions revealed a decrease of 88 % between 1970 and 1995. While in 1970 the total HCB emission was estimated to be 192 tonnes/year, the corresponding level in 1995 amounted to 23 tonnes/year (Eurochlor, 2005). It can be estimated that the HCB emissions have further declined over the past 10 years as exemplified by a recent HCB inventory from Austria, which revealed that there had been a reduction of total HCB emissions of 50 % for the period 1990 to 2003. The total emission in the year 2003 was estimated to be around 47 kg (Austrian Environmental Authority, 2005). This substantial reduction of HCB emissions can certainly be attributed to the significant improvement and optimisation of incineration technology. In a recent investigation performed by the Swedish

Environmental Protection Agency (2005), the following national annual releases from a few sectors are reported: combustion 1.5 - 9.6 kg, chemical industry 12.5 kg and shipping 0.8 - 2.0 kg.

1.2.2. Environmental fate

HCB is among the more persistent environmental pollutants and, when released into the atmosphere, it can exist in both the vapour phase and in association with particles (Eisenreich *et al.,* 1981). However, monitoring studies have demonstrated that the vapour phase generally predominates (Ballschmiter and Wittlinger, 1991; Bidleman *et al.,* 1989; Lane *et al.,* 1992).

In the troposphere, HCB is transported over long distances from the temperate to the polar-regions by virtue of its persistence and relatively high vapour pressure, but does undergo slow photolytic degradation with an estimated half-life of approximately 80 days (Mill and Haag, 1986). The photo-oxidation half-life (first-order kinetics) of HCB based on the vapour phase reaction with hydroxyl radicals in air, was estimated to range from 0.43 to 4.3 years by Howard *et al.* (1991) and Kwok and Atkinson (1995). Wania and Mackay (1995) estimated the degradation half-life (first-order kinetics) of HCB to be 0.63, 1.94 and 6.28 years in air in tropical/subtropical regions, temperate/boreal regions, and polar-regions, respectively. Brubaker and Hites (1998) measured a hydroxyl rate constant of 2.7 x 10^{-14} cm^3/molecule-second at 25ºC, corresponding to a calculated half-life of 1.69 years. Thus, atmospheric degradation of HCB is extremely slow.

In water, HCB is not readily degraded by either abiotic or biotic processes. It is resistant to the types of hydrolysis reactions that can degrade other organochlorines or organophosphates, and it is not markedly subject to photolytic decay (Mill and Haag, 1986). The half-life value of HCB is estimated to range from 2.7 to 5.7 years in surface water and from 5.3 to 11.4 years in groundwater. Volatilisation from the water column is moderately rapid; however, the compound's strong adsorption to particulates and organic matter in water can result in long persistence in the sediment. When released into soil, HCB will be strongly adsorbed to organic matter and is generally considered immobile with respect to leaching. Its half-life value in soils is estimated to be in the range of three to six years. HCB is significantly bioaccumulated in both terrestrial and aquatic food chains and a bioconcentration factor as high as 21,900 has been reported for fish by Veith *et al.* (1979). Both the bioconcentration factor and elimination half-life ($t_{½}$) seem to increase with increasing concentration in exposure medium (Giam *et al.,* 1980).

Volatilisation from water to air and sedimentation following adsorption to suspended particulates are the major removal processes from water (Oliver, 1984a; Oliver and Charlton, 1984). Once in the sediments, HCB will tend to accumulate and become trapped by overlying sediments (Oliver and Nicol, 1982). Although HCB does not readily leach from soils and sediments, some desorption does occur and these could therefore act as continuous sources of HCB to the environment (Oliver, 1984a; Oliver *et al*., 1989). Chemical or biological degradation is not considered to be important for the removal of HCB from water or

sediments (Callahan *et al.,* 1979; Mansour *et al.,* 1986; Mill and Haag, 1986; Oliver and Carey, 1986). In soil, volatilisation is the major removal process at the surface (Kilzer *et al.,* 1979; Griffin and Chou, 1981; Schwarzenbach *et al.,* 1983; Nash and Gish, 1989), while slow aerobic (half-life of 2.7 - 5.7 years) and anaerobic biodegradation (half-life of 10.6 - 22.9 years) are the major removal processes at lower depths (Beck and Hansen, 1974; Howard *et al.,* 1991). Pumpkins, belonging to the *Cucurbitaceae,* have been shown to be able to absorb and distribute HCB via the root system and accumulate it in their fatty seeds (see chapter 4).

Field studies indicate that oral exposure is important for organisms at higher trophic levels, as significant biomagnification has been observed in several studies in natural aquatic ecosystems. The bioaccumulative properties of HCB result from the combination of its physicochemical properties (high octanol/water partition coefficient) and its slow elimination due to limited metabolism related to its high chemical stability. Organisms generally accumulate HCB from water and from food, although benthic organisms may also accumulate HCB directly from sediment (Oliver, 1984b; Knezovich and Harrison, 1988; Gobas *et al.,* 1989).

1.3. Toxicology in laboratory animals and hazard assessment for humans

The toxicology of HCB was reviewed by WHO in 1997 (WHO-IPCS, 1997), IARC in 1979 and 2001 (IARC 1979, 2001) and the Agency for Toxic Substances and Disease Registry in 2002 (ATSDR, 2002).

HCB has low acute toxicity in experimental animals with LD_{50} values being in the range of 1000 to 10,000 mg/kg b.w. per day for various species. Acute lethal doses trigger convulsions, tremor, ataxia and paralysis. At lower doses the major target organ of HCB toxicity in laboratory experimental animals and in humans is the liver and signs include porphyria. Other targets are the nervous system, skin, bone and thyroid gland, but symptoms from these organs have been reported less frequently than porphyria.

The major biochemical effect is inhibition of uroporphyrinogen decarboxylase in the haem biosynthetic pathway, which causes elevated levels of porphyrins and/or porphyrin precursors (porphyria). Porphyria has been reported in a number of studies in rats with sub-chronic or chronic oral exposure to doses between 2.5 and 15 mg HCB/kg b.w. per day. Female rats are more sensitive than male rats to the porphyrogenic effects on the liver.Although the mechanism remains to be clearly defined, results from mechanistic studies suggest that oxidative metabolism of HCB plays a crucial role in the development of porphyria. In female rats, Van Ommen *et al.* (1989) observed that the urinary excretion of the major oxidative metabolites of HCB, pentachlorophenol (PCP) and tetrachloro-1,4-hydroquinone as well as the extent of hepatic accumulation and excretion of porphyrins, were greatly reduced in animals co-treated with triacetyloleandomycin (a selective inhibitor of the cytochrome P450 isoforms CYP3A1 and CYP3A2) compared with animals given HCB alone. This result was confirmed by Den Besten *et al.*, 1994. Another biochemical effect of HCB is mixed-type cytochrome-P450-induction similar to that obtained by combined exposure to phenobarbital

and 3-methylcholanthrene. HCB is reputed to be able to bind to the Ah receptor with very low affinity (Van Birgelen, 1998; Miller, 1999). However, it cannot be excluded that this was due to contamination with higher chlorinated dioxins and furans. Ah-responsive mice were more sensitive than non-responsive mice to HCB induced porphyrin accumulation in the liver. Subchronic HCB exposure (0.7 mg/kg b.w.) caused disturbed calcium homestasis and bone morphometry in rats, which showed reduced calcium excretion and elevated serum levels of 1,25-dihydroxy-vitamin-D3. Increased elimination of thyroxin (T4) and triiodothyronine (T3) followed by decreased levels of T4 and T3 are accompanied by increased synthesis of thyroid stimulating hormone (TSH) and compensatory growth of the thyroid gland.

In humans, manifestations of disturbances in porphyrin metabolism were observed in accidental poisonings that took place in Turkey from 1955 to 1959. It was estimated that as many as 3 - 5000 persons were affected. More than 600 cases of porphyria cutanea tarda were clinically identified. Symptoms observed were dermatological lesions, including erythema, bullae, ulcerations and scarring, hyperpigmentation and hypertrichosis, enlarged liver, enlargement of the thyroid gland and lymph nodes. Among roughly half the cases, primarily in children, osteoporosis or arthritis was also observed. No exposure data were given in clinical reports (ATSDR, 2002). In this incident children < 4 years rarely developed porphyria cutanea tarda, but breastfed infants of mothers exposed to HCB developed a disorder called pembe yara (pink sore, characteristic pink cutaneous lesions), and most infants died within a year. There is also limited evidence that porphyria cutanea tarda occurs in humans with high exposure to HCB in the workplace or in the general environment (WHO-IPCS, 1997, IARC 2001).

Due to accumulation, the doses resulting in effects are much lower following long term exposure than those from acute or short term dosing. At chronic exposures between 0.25 and 0.6 mg HCB/kg b.w. per day, rats showed mild effects in the liver (proliferation of smooth endoplasmatic reticulum, altered mitochondria and increased number of storage vesicles, enzyme induction); the ‘no observed adverse effect levels’ (NOAELs) in these studies were 0.05 to 0.07 mg HCB/kg b.w. per day. Changes in bone structure associated with disturbed calcium homoeostasis were observed in sub-chronic studies in rats at 0.7 mg HCB/kg b.w. per day, but not at 0.07 mg/kg b.w. per day. The lowest NOAEL, 0.05 - 0.07 mg/kg b.w. for hepatic effects were found in a subchronic study in pigs (see section 5.4) and several chronic studies in rats WHO-IPCS 1997). Beagle dogs (see section 5.7) were much less sensitive to hepatotoxicity

1.3.1. Immunotoxicity

Several studies in rats, beagle dogs and monkeys show that HCB affects the immune system and that it could cause both suppressive and stimulating effects at doses of 0.5 - 20 mg/kg b.w. per day for several weeks. Manifestations are histopathological alterations in the thymus, spleen, lymph nodes and/or lymphoid tissues of the lung in rats and monkeys, whereas in beagle dogs HCB caused nodular hyperplasia of the gastric lymphoid tissue. Humoral

immunity and, to a lesser extent, cell-mediated immunity were enhanced, while macrophage function was unaltered following HCB exposure in rats. Perinatal HCB exposure increased humoral and cell-mediated immune responses and caused accumulation of macrophages in the lung tissue of rat pups, but was immunosuppressive in most studies with mice. The Brown Norway rat is particularly susceptible to HCB induced immunopathology consisting of inflammatory responses in skin, lung, lymph nodes, eosinophilia and increased serum levels of IgE and anti-single stranded DNA IgM (Ezendam *et al.*, 2005). Macrophages appear to play a central role. Up-regulation of genes associated with processes known to be induced by HCB such as stimulation of the immune system and production of pro-inflammatory cytokines, induction of drug metabolising enzymes, porphyria and effects on reproduction were seen in Brown Norway rats dosed 150 - 450 mg HCB/kg feed for several weeks (15 - 45 mg HCB/kg b.w. per day) (Ezendam *et al.*, 2004).

1.3.2. Reproductive toxicity

In monkeys orally exposed to doses as low as 0.1 mg HCB/kg b.w. per day for 90 days, steroidogenesis and changes in the surface of the ovarian germinal epithelium as well as ultra-structural injury to the primordial germ have been observed. These specific target sites, which are damaged further at higher doses, were associated with otherwise normal follicular, oocyte and embryo development, suggesting specificity of HCB action within the site of the ovary. Male reproductive functions as studied in mice, rats and pigs were only affected at much higher doses (e.g. 30 mg/kg b.w. per day for 21 days in mice, 50 mg/kg b.w. per day for 90 days in pigs and 221 mg/kg b.w. per day for five days in rats). Transplacental or lactational HCB exposure of rats and cats was hepatotoxic and/or affected the survival or growth of suckling offspring at doses between 1.5 to 4 mg/kg b.w. per day. Reduced litter sizes and/or increased numbers of stillbirths could also be observed at these or higher doses. Adverse effects on suckling offspring have generally been observed more frequently, and at lower doses, than embryotoxic or foetotoxic effects. Skeletal and renal abnormalities observed in foetuses in some studies of rats and mice exposed to HCB during gestation occurred at doses that were also maternally toxic. Structural malformations in mice exposed *in utero* to HCB were strikingly like those of 2,3,7,8-tetrachlorodibenzo-*para*-dioxin (TCDD), which has been observed as a contaminant in technical HCB. Also, the neurobehavioral development of rat pups was affected following *in utero* exposure to HCB at maternal doses as low as 0.64 mg/kg b.w. per day 90 days prior to mating and throughout gestation and lactation (IARC, 2001; WHO-IPCS, 1997).

1.3.3. Genotoxicity

Cytochromes P450 mediated oxidation of HCB can result in the formation of electrophilic intermediates such as epoxides and/or benzoquinones, which can covalently bind with proteins and DNA (Rietjens *et al.*, 1997). In a study to test the DNA-binding of HCB, untreated or phenobarbital pre-treated male Wistar rats were administered 25 mg/kg

radiolabelled HCB in refined peanut oil for 24 hours (Gopalaswamy and Nair, 1992). The animals were sacrificed and DNA obtained from liver extracts. Upon analysis, HCB DNA-binding was found to be 2.23 ± 0.27 pmoles/mg DNA for phenobarbital untreated animals and 3.56 ± 0.18 for phenobarbital pre-treated animals

In vitro, specific binding of HCB to DNA was only slightly above background in hepatocytes isolated from phenobarbital-treated rats (Stewart and Smith, 1987).

Studies on the evaluation of genotoxicity of HCB are limited but in general they show a lack of evidence of mutagenicity, chromosomal damage or unscheduled DNA repair. Only in a small number of studies on bacteria and yeast HCB exhibited weak mutagenic activity (WHO-ICPS, 1997; IARC, 2001). In one study HCB (at doses of 0.1 - 0.56 mM) induced micronuclei in rat and human hepatocytes (Canonero *et al.*, 1997).

1.3.4. Carcinogenicity

Animals

Several studies on the carcinogenicity of HCB have been carried out in rodents. In hamsters, liver tumours (hepatomas) in both sexes, and haemangioendotheliomas of the liver and thyroid follicular cell adenomas in males were observed. Hepatomas were also found in mice after HCB treatment. Studies on rats exposed to HCB perinatally and/or in diet for two years or more had increased incidences of hepatomas, hepatocellular carcinomas, bile duct adenomas/carcinomas, renal tumours, adrenal phaeochromocytomas and parathyroid adenomas. The doses inducing tumours were in the order of 25 mg/kg b.w. per day. HCB promoted liver tumours after induction in mice and rats. Renal tumours in male rats appear - at least in part - to be the result of hyaline droplet nephropathy, a mode of action which is not considered relevant for humans (Bouthillier *et al.,* 1991). Thyroid tumours in rats could be caused by the hypothyroid effect of HCB followed by TSH stimulation of the thyroid gland. Hepatomas in rats may result from enzyme induction, iron accumulation, oxidative damage, and hyperplastic responses to HCB. Otherwise, mechanistic studies addressing the relevance of the tumour types induced by HCB for humans have not been identified (IARC, 2001; WHO-IPCS, 1997).

Humans

Five small and four large scale studies investigating the risk of breast cancer in relation to life-long exposure to HCB were evaluated by IARC (2001). As HCB accumulates over time, it is difficult to interpret studies that are not strictly controlled for age. Furthermore, the numbers of breastfed children and length of the breastfeeding period(s) considerably influence the HCB levels. In one of the small studies (Liljegren *et al.,* 1998), a secondary sub-group analysis showed a significant association between HCB and risk in post-menopausal women with estrogen receptor-positive cancer. In one of the larger studies (Dorgan *et al.,* 1999) the risk of breast cancer in women with HCB levels in the serum in the upper three quartiles was twice those of women whose levels were in the lower quartile. However, this was observed

only in women whose blood was collected close to the time of diagnosis and no dose-response relationship was found. In two recent European case-control studies Charlier *et al.* (2003, 2004), the HCB level in serum was significantly higher in women with breast cancer than controls, but HCB was only detected in 10 - 20 % of the samples. The Panel noted that the low fraction of samples positive for HCB in these studies weakens the association of HCB as a causal agent.

Grimalt *et al.* (1994) reported a study on cancer incidence (129 cases in all) among the inhabitants of Flix, Spain, a small village in the vicinity of an electrochemical factory producing chlorine and organochlorine compounds. The factory had been producing chlorinated solvents for four decades, and also DDT and PCBs of which production ended in 1971 and 1987, respectively (Ribas-Fito, 2003). The major source of HCB in the Flix area is the industrial emission as a side-product related to the manufacture of chlorinated solvents. Air levels of HCB observed in 1994, were higher in Flix (35 ng/m^3) than in nearby Barcelona (0.3 ng/m^3). The mean HCB level in serum from village inhabitants was 26 µg/L, whereas the mean level from the reference population (samples collected from a hospital in Barcelona) was 4.8 µg/L. Compared with the cancer incidence rates for the province as a whole, there was a significant excess of thyroid neoplasm and soft-tissue sarcoma in men (ATSDR, 2002). The Panel noted that the reported increases were based on only two and three cases, respectively. The population studied was also exposed to several other organochlorine compounds, but the relative levels were much lower than those of HCB. In addition, a cross-sectional study was also performed in Flix (Herrero *et al.*, 1999). Although elevated HCB levels were detected in all serum samples (values ranging from 1.1 to 1616 ng/mL, mean = 40 ng/mL), there was no association between the HCB concentrations and porphyrin levels for the 604 study participants.

1.3.5. Evaluations

IARC classified HCB as possibly carcinogenic to humans (Group 2B, inadequate evidence in humans and sufficient evidence in experimental animals) in 2001 (IARC, 2001).

In 1997, IPCS suggested a health based guidance value of 0.17 µg/kg b.w. per day based on hepatic effects, ultrastructural changes in rats and increased urinary coproporphyrin and microsomal liver enzyme activity in pigs, and by incorporating an uncertainty factor of 300 (10 for interspecies variation, 10 for intra-species variation, and 3 for severity of effect). They also calculated a tumour dose 5 % (T5) (i.e., the intake associated with a 5 % excess incidence of tumours in experimental studies in animals) of 0.81 mg/kg b.w. per day based on incidence of neoplastic liver nodules in females of the two-generation carcinogenicity bioassay in rats. Based on this dose IPCS developed a "Health based guidance value for cancer risk (WHO-IPCS, 1997). EFSA does not derive health based guidance values for compounds that are both carcinogenic and genotoxic, but uses a margin of exposure approach (MOE) comparing the dose descriptor BMDL10 (the lower confidence level of the dose associated with a 10 % excess incidence of tumours in experimental studies in animals) with the actual exposure levels (see Conclusions, Effects on humans and human exposure).

2. Methods of analysis

A number of well-proven, validated multi-residue methods for the quantitative determination of HCB as well as other organochlorine pesticides in various matrices including food, feed and other biological specimens are available. Depending on the type of feed material - whether of plant or animal origin - extraction of HCB and other persistent organic pollutants, as well as the extent of necessary subsequent clean-up steps, may differ considerably. Solid materials are commonly extracted, after grinding, with boiling organic solvents using conventional Soxhlet, accelerated solvent or microwave assisted extraction procedures or by supercritical fluid extraction. On the other hand, liquid samples are mostly extracted by liquid/liquid partitioning. Co-extracted fat and other compounds which potentially may disturb the HCB determination can be removed by gel permeation chromatography or by adsorption chromatography on various solid phase materials. Due to the high electro negativity caused by the six chlorine atoms of the compound, high-resolution gas chromatography with electron capture detection (HRGC/ECD) is the analytical method widely used to separate HCB from possible interfering co-extractants and to detect it with high sensitivity.

An efficient separation of HCB from other interfering compounds, such as other organochlorine pesticides and polychlorinated biphenyls (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 coelution problems can also be overcome by applying combined high resolution gas chromatography/mass spectrometry (HRGC/MS) either in the electron impact or negative chemical ionization mode. The latter ionization technique is highly sensitive and allows the determination of HCB down to the femtogram range. Besides 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 determination and behave as the native analytes, they allow a reliable overview of the losses during the analytical procedure and thus significantly increase the accuracy of the results. Multi-residue procedures for PCBs and pesticides including HCB using HRGC/ECD and 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 limit of quantification for HCB is given as 2 and 0.5 ng/g, respectively.

3. Statutory limits

The use of HCB as a pesticide has been banned in the EU since 1981 by Directive 79/117/EEC[16] which prohibits the placing on the market and use of plant protection products containing certain substances.

HCB is listed in the Annex to Directive 2002/32/EC on undesirable substances in animal feed[17] which replaced Directive 1999/29/EC on the undesirable substances and products in animal nutrition[18] on 1 August 2003. The maximum levels pertain to a feedingstuff with a moisture content of 12 %. The maximum levels in feedingstuffs are comparable with those established in food (see also background, chapter 2).

Neither MRLs nor extraneous maximum residue limits (EMRL) have been established by the Codex Alimentarius Commission[19].

4. Occurrence in feed and animal exposure

HCB 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.

Feed materials of plant origin are predominantly contaminated with organic pollutants through air by gas or particle phase deposition. Therefore, vegetables and crops with large and waxy leaf surfaces grown in areas with elevated HCB emissions are more likely to be contaminated. In contrast, uptake of persistent organic pollutants by roots is generally low due to their low water solubility. However, dioxins and HCB have been shown to be taken up by roots. Hülster *et al.* demonstrated the considerable uptake of dioxins by zucchini via roots and subsequent translocation to the shoots (Hülster *et al.,* 1994). Oil pumpkins have a special

[16] OJ L 33, 8.2.1979, p. 36
[17] OJ L140, 30.5.2002, p. 10
[18] OJ L 115, 4.5.1999, p. 32
[19] http://faostat.fao.org/faostat

ability for root uptake of HCB and its translocation into shoots during different growth stages as reported by Ecker and Horak (Ecker and Horak, 1994). They found a positive correlation between the HCB soil concentration and the seed oil of pumpkins grown on this soil. The HCB concentrations ranged from 1.3 to 11.4 µg/kg soil and from 20 to 280 µg/kg seed oil. In contrast, increasing lindane (gamma-HCH) values in these soils had no significant effect on the lindane concentrations in the pumpkin seeds. Recent investigations of pumpkin seeds/oil produced in Austria confirmed the extraordinary ability to accumulate HCB in the seeds. Between 2000 and 2004 a total of 675 pumpkin seed oils were analysed for HCB and the results converted to residues in pumpkin using an empirically derived conversion factor of 2.3. While in 51 % of all samples the HCB level was below 10 µg/kg, 0.6 % of the samples contained more than 200 µg/kg and the highest amount was found to be 1450 µg/kg (AGES, 2005).

In Belgium a total of 871 single and compound feed samples were collected and analysed between 2000 and 2004. Four samples contained HCB at concentrations between 1 and 14 µg/kg. HCB could not be determined in any of the other samples at a reported limit of quantification of 2 µg/kg. In Estonia 42 feed samples, mainly grain and complete feedingstuffs, were analysed between May 2004 and March 2005. HCB could only be detected in one rape cake sample at the limit of detection of 10 µg/kg product. Denmark reported results on undesirable substances in 994 feed samples collected and analysed from 1998 to 2004. Only one complete feedingstuff mix for fish contained HCB at a level of 2 µg/kg. Germany reported on the results of 290 feed samples of plant origin collected and analysed in 2004 for undesirable substances. None of these samples, comprised mainly of soy bean products, citrus pulp pellets, corn pellets and palm kernel derived products, contained HCB above the limit of detection of 5 µg/kg.

Extensive monitoring programs for HCB and other organochlorine pesticides were performed by the State Veterinary Administration of the Czech Republic between 2002 and 2004. In 2004 a total of 200 complete and complementary feed samples were analysed. 44 of these samples contained HCB. The maximum concentration was found to be 10 µg/kg. 132 out of 470 complete feed samples analysed in 2002/2003 were found to be positive for HCB with a maximum of 153 µg/kg. In the same period 18 feeding cereals (five positive results with a maximum level of 26 µg/kg), 112 feeding raw materials of animal origin (54 positive results with a maximum level 2 µg/kg) and six mineral feeds (two positive results with a maximum level 2 µg/kg) were analysed for HCB and other undesirable substances. Moreover, the Czech State Veterinary Administration reported on the results of 221 fish meal samples analysed between 2002 and 2004. HCB was found in 102 of these samples with a maximum concentration of 2 µg/kg. Details on the composition of the individual contaminated samples were not given, and neither were the ranges of concentration nor the limit of quantification for the different feed commodities provided. Additional information from the Czech Republic (Ruprich, 2006, unpublished data) gives detailed results for 16 fish meal samples collected in 2004 and 10 fish meal samples collected in 2003. In these feeding stuffs the HCB levels ranged between < 0.5 and 2.7 and 1.1 - 3.6 µg/kg, respectively. In the year 2003 five meat and bone meal samples were also collected and analysed. HCB concentrations were reported to be

1.1 - 2.8 µg/kg. In Finland 14 feed samples of plant and animal origin were analysed recently for undesirable substances. In all samples, HCB was below the limit of determination. This limit was 10 µg/kg except for cod liver oil (one sample) which had a limit of determination of 200 µg/kg, all based on 12 % moisture content.

Norway reported on the results of HCB occurrence in 34 feed samples analysed in 2004. The levels ranged between 0.2 and 15 µg/kg. The highest levels were found in six fish oil samples (0.9 - 15 µg/kg) and in compound feed for fish (0.9 - 4.2 µg/kg). With one exception (vegetable oil, 2.6 µg/kg) the concentration of HCB was found to be < 1 µg/kg in all other samples of plant origin. The limit of detection was 0.04 µg/kg. The HCB levels measured in 41 feed samples in 2005 revealed levels in a comparable range. In fish oil (n = 10) the maximum level was found to be 4.0 µg/kg.

In Iceland 30 fish meal and 21 fish oil samples were analysed for HCB and other pesticides in 2003/2004 as part of a monitoring programme on undesirable substances in seafood products. The corresponding HCB levels ranged from < 0.6 to 4.1 and 8.3 to 57 µg/kg, respectively, each based on a moisture content of 12 % (Icelandic Fisheries Laboratories, 2004, 2005).

Recent data on 16 fish feed samples for salmonids provided by the European Feed Manufacturers' Federation, showed no HCB at a limit of detection of 5 µg/kg.

Although interpretation of the occurrence data of the various feed commodities are hampered by the large differences in the respective limits of determination, the data indicate that feed materials of animal origin, such as fish derived products, are generally slightly more contaminated than feed materials of plant origin with the exception of pumpkin seeds. However, the contamination of fish with HCB does not seem to be as prominent as with other organohalogen contaminants. This is substantiated by recent investigations in Germany and Sweden. In Germany 443 farmed fish (carp, rainbow trout, salmon) and 182 wild fish (herring, mackerel, pilchard anchovy, tuna) samples were analysed recently for HCB and a number of other contaminants. These investigations were carried out within the framework of official food controls and a monitoring programme. Median HCB levels in all species were generally < 1 µg/kg fresh weight, except for salmon which had a median HCB concentration of 1.6 µg/kg fresh weight. Similar results were found in Sweden and Iceland. In Sweden 446 herring samples from the Baltic Sea were analysed between 2001 and 2003. The median value was found to be 2.4 µg/kg fresh weight. Within the above mentioned Icelandic monitoring programme the HCB levels in 60 fish and seafood samples, each containing 10 individuals, ranged from 0.05 to 3.7 µg/kg fresh weight (Icelandic Fisheries Laboratories, 2004).

As POPs are environmental contaminants with a strong potential for bioaccumulation, these chemicals are expected to be present in farm animals and food products of animal origin which represent the predominant source for human exposure (Weiss *et al.*, 2005). Analysis of butter samples has proven to be an effective means of assessing the global contamination of persistent organic pollutants. Butter may be seen as a representative sample type for several dairy products, because it is a homogeneous, fat-rich matrix usually composed of milk fat from numerous farms. The results of the analyses of 64 butter samples from 37 countries for

HCB are depicted in Figure 2 (Weiss *et al.*, 2005). The data indicate the wide range of HCB levels in butter from different countries. The highest concentrations were found in butter samples originating from Russia, Ukraine and Slovenia. The lowest concentrations were found in samples from Thailand and Malaysia. Although not claiming to be representative, because for most countries only one sample was analysed, nevertheless these results give some indication of those countries where elevated HCB pollution exists and consequently one has to consider that feed materials produced in the respective areas might also contain elevated HCB levels.

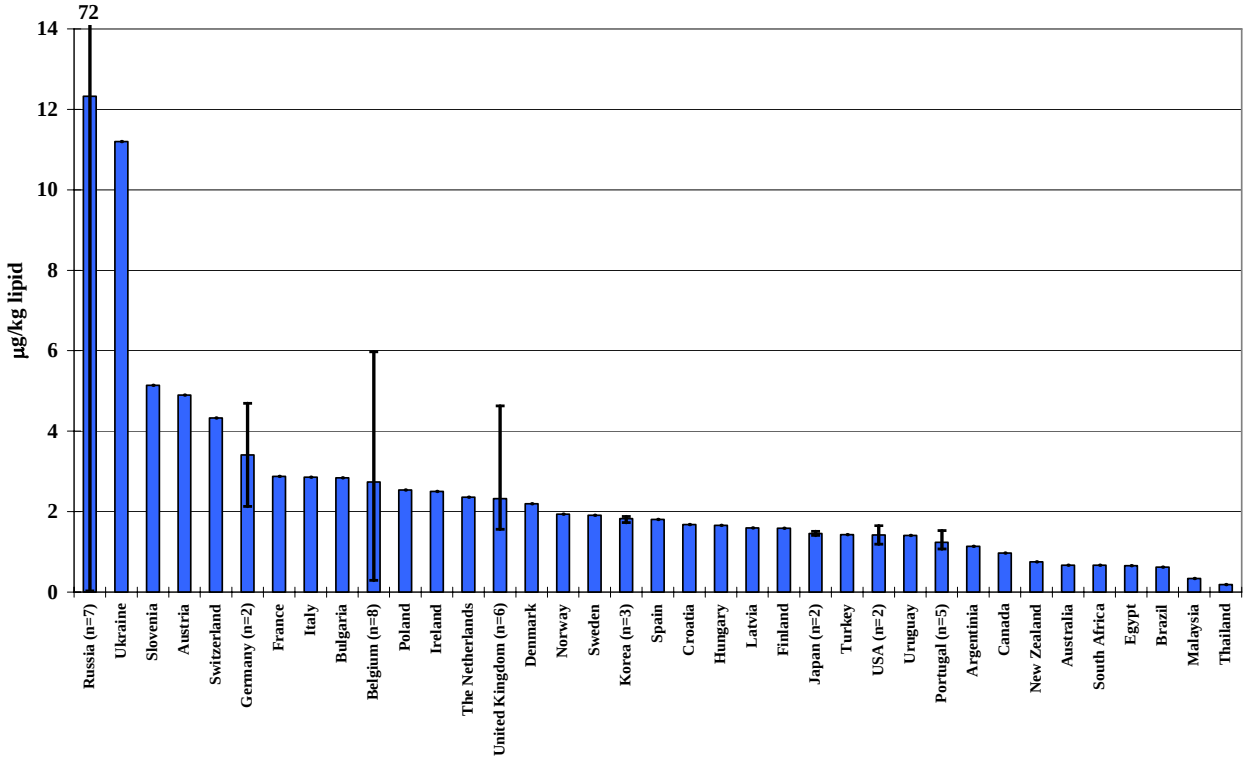

Figure 2. Concentrations (µg/kg lipid) of HCB in butter samples from various countries, levels are for one sample if range is not given (data from Weiss *et al.*, 2005).

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

5.1. Introduction

Fish and terrestrial animals may be exposed to HCB through contaminated diet. The compound show higher toxicity when administered in oil, particularly in vegetable oil, than in other matrices (Koss and Koransky, 1975). Fish may also be exposed via water and sediment.

The sensitivity to HCB exposure varies with species, strain, age, gender, health status and fat depot. Since HCB is deposited and to a large extent immobilized in fat depots, animals with relatively small fat depots are generally more susceptible to poisoning than fat animals. The compound may be redistributed and become available to target tissue during emaciation.

HCB shows a relatively low acute toxicity as compared with other organochlorine pesticides and risk is associated with chronic toxicity due to prolonged exposure and accumulation. Hepatotoxicity including porphyria and liver enzyme induction as well as neurotoxicity, and reduced body weight gain are the prominent effects after sub-chronic feeding of HCB (Booth and McDowell, 1975; Strik, 1986).

Symptoms of acute intoxication are convulsions, tremors, weakness, ataxia, paralysis and pathological changes in organs (Strik, 1986). Acute oral toxicity, LD_{50}, varies from > 1000 to > 10,000 mg/kg b.w. per day in different species (see chapter 1.3).

Most technical grade HCB products contained impurities. Pentachlorobenzene was the major contaminant. Other compounds discovered included hepta- and octachlorodibenzofuran and octachlorodibenzo-p-dioxin (see chapter 1.1). The contaminants may contribute to the overall toxicity of the products (Villanueva *et al.,* 1974).

5.2. Fish

Sub-adult rainbow trout were fed low levels of HCB, up to 0.78 mg/kg feed for 57 days or up to 7 mg/kg feed (0.06 mg/kg b.w. per day) for 28 days in studies focusing on toxicokinetics. No effects were found on body weight gain during the study. Other toxicity endpoints were not examined (Niimi and Cho, 1980; 1981).

In short- or longer term experiments with fish exposed to HCB in water, no mortality or effects on growth after exposure to levels approaching its aqueous solubility were found (WHO, 1997).

5.3. Ruminants

For five days a week over an 18 week period, one-year-old female sheep were given an oral dose of the commercial fungicide Voronit® powder in gelatine capsules with 60 % HCB at 0, 0.1, 1.0, 10 or 100 mg HCB/sheep per day, (Avrahami and Steele, 1972a). As well as HCB, Voronit® was also reported to contain 10 % 2-(2´furyl)-benzimidazole, 10 % benquinox, together with inert fillers and red pigment. The HCB doses per animal correspond approximately to 0.004, 0.04, 0.4 and 4 mg HCB/kg b.w. per day, and 0.1, 1, 10 and 100 mg HCB/kg diet. The main purpose was to study the storage and elimination of HCB, but the body weight was also recorded at weekly intervals. Reduced weight gain was found at the highest dosage level only. A possible contribution from the other compounds to the effects was not discussed by the authors.

Growing castrated male lambs were fed HCB (purity >99.5 %) at 0, 0.01, 0.1 or 1.0 mg/kg diet for 90 days, or at 100 mg/kg diet for 19 days (Mull *et al.,* 1978). Growth rates (90 day exposure), certain plasma enzyme activities and hepatic microsomal enzyme activities, plasma proteins, hematocrit, and histopathology were determined. *In vivo* metabolism of antipyrine and hepatic microsomal N-demethylase were significantly increased at 100 mg/kg

diet, and the latter also at 1 mg/kg diet. Hepatic microsomal O-demethylase was significantly increased only after the 1 mg/kg exposure. A concentration of 0.1 mg/kg diet fed to lambs did not show any effects on hepatic enzymes, and a NOEL of 0.004 mg/kg b.w. per day can be derived from this study.

5.4. Pigs

Crossbred weanling gilts were fed a standard ration containing 0, 1, 10 or 100 mg of purified HCB/kg diet (corresponding to approximately 0.04 - 0.06, 0.4 - 0.6, and 4 - 6 mg/kg b.w. per day, respectively) for 13 weeks (Hansen *et al.,* 1977). Clinical, biochemical, haematological, gross- and micropathological effects were examined. Post mortem examination under ultraviolet light revealed brightly fluorescent livers (due to porphyrin accumulation) at all dosage levels of HCB. At a dietary level of 100 mg/kg enlarged livers with swollen hepatocytes and lymphopenia were observed. The lowest concentration (1 mg/kg diet) given to gilts showed an adverse effect, and a LOAEL of 0.04 mg/kg b.w. per day can be derived from this study.

In another study, weanling male pigs were fed HCB at doses of 0.05, 0.5, 5.0 and 50 mg/kg b.w. per day (corresponding to 0.8 - 1.3, 8 - 13, 80 - 130 and 800 - 1300 mg/kg diet, respectively) for 13 weeks (den Tonkelaar *et al.,* 1978). Special attention was given to the induction of porphyria and microsomal liver enzymes and tissue levels of HCB. Animals at the highest dose showed clinical signs associated with porphyria and died during the experiment. At lower dosages these signs were not observed. Increased liver weight were found at 5 mg/kg b.w. per day. Histopathological changes in the liver and induction of hepatic microsomal enzymes as well as increased excretion of coproporphyrin was found at 5 and 0.5 mg/kg b.w. per day. From this study a NOAEL of 0.05 mg/kg b.w. per day can be derived.

Third-litter sows were fed purified HCB at concentrations of 0, 1 or 20 mg/kg diet (corresponding to 0.025 and 0.5 mg/kg b.w. per day) throughout the breeding, gestation and nursing phases up to weaning at six weeks post partum. The main purpose was to examine the HCB distribution (Hansen *et al.,* 1979a). Clinical, reproductive, haematological, gross- and histopathological effects were also examined in sows and piglets. The authors reported no adverse effects on the sows or piglets. Effects were found in some of the HCB treated sows, but it was uncertain whether they related directly to HCB exposure. The effects ranged from a catarrhal exudation to mild ulceration in the stomach, a mildly decreased splenic white pulp, renal interstitial fibroplasia, fatty replacement of Brunner´s gland in duodenum to pancreatic periductal fibrosis. In the offspring exposed to HCB, a dose related increase of erythrocyte volume was found.

5.5. Birds

Growing chickens (from age 12 days) were fed HCB at 0, 0.1, 1, 10 or 100 mg/kg diet for six months (Avrahami and Steele, 1972b), in the form of a commercial fungicide powder

(Voronit®), containing 60 % HCB, 10 % 2-(2´furyl)-benzimidazole, 10 % benquinox, together with inert fillers and red pigment. Body weight, liver weight and tissue accumulation of HCB were studied. No adverse effects on the general health, body weight gain or liver weight were found.

Broiler cockerels (eight days old) received HCB (purity >99 %) at 0, 1, 10 or 100 mg/kg diet for 25, 38 or 52 days (Hansen *et al.,* 1979b). Body weight, haematology, gross and histopathology and HCB residues were determined. Birds treated at the two highest dosage levels grew significantly faster for the first 25 days, but slower later on. No difference in net weight was observed at 52 days. Birds at 100 mg/kg diet had enlarged adrenal glands and livers. A concentration of 10 mg/kg diet fed to broiler cockerels did not show any adverse effects, and a NOAEL of 1 mg/kg b.w. per day can be derived from this study.

HCB was fed to eight month old laying hens at 0, 0.1, 1, 10, or 100 mg/kg diet for six months to study the effect on egg production, hatchability and tissue accumulation of HCB (Avrahami and Steele, 1972c). The fungicide powder Voronit®, containing 60 % HCB, 10 % 2-(2´furyl)-benzimidazole, 10 % benquinox, together with inert fillers and red pigment was added to the feed. The feeding appeared to have no adverse clinical effects on the health neither of the hens or the chickens nor on the fertility or hatchability of the eggs (Avrahami and Steele, 1972c). A concentration of 100 mg/kg diet laying hens did not show any adverse effects, and a NOAEL of 6 mg/kg b.w. per day can be derived from this study.

20-week-old pullets were given daily oral doses of HCB ranging from 1 to 100 mg/kg b.w. per day for seven days (Hansen *et al*., 1978). HCB treatment at 10 mg/kg b.w (corresponding to 125 mg/kg b.w. per day) and above was found to delay the onset of full egg production. No effects on body weight, feed consumption or adverse effects on gross pathology were observed.

In another study on laying hens, HCB was given at 125 or 625 mg/kg feed (corresponding to 8 and 40 mg/kg b.w. per day) for 12 weeks (Kan *et al.,* 1979). The parameters studied were body weight, feed consumption, egg production, quality of egg shell, porphyrin excretion, liver, kidney, gall bladder, and spleen weight, and histopathology of liver, kidney and thyroid, as well as the activity of drug enzymes in liver. Both dosage levels induced the hepatic enzyme activity (aniline hydroxylase, N-demethylase, cytochrome P-450 content). The highest dosage level also decreased the feed consumption and the body weight and increased the relative liver weight. No other treatment related effects were found. The lowest concentration (125 mg/kg diet) given to laying hens showed an adverse effect, and a LOAEL of 8 mg/kg b.w. per day can be derived from this study.

The oral LD_{50} for HCB in Japanese quail is > 6400 mg/kg b.w. per day (Strik, 1986).

Adult Japanese quail were fed HCB at concentrations of 0, 20, 100, 500 or 2500 mg/kg diet for up to three months (Vos *et al.*, 1968). Birds at the two highest dosages died within a month after weight loss, apathy and neurological symptoms. Most of the birds that received 100 mg/kg died within seven weeks. Fluorescence microscopy revealed significant porphyrin

accumulation at the three highest dosages. All animals fed 20 mg/kg diet survived and did not develop visible symptoms. However, they showed reduced hatchability of the eggs, a tendency towards fatty infiltration of the liver and an accumulation of porphyrins. In a follow-up study, Vos *et al.* (1971) fed Japanese quail aged two to five months HCB (purity ≥99.5 %) at 0, 1, 5, 20 and 80 mg/kg diet for 90 days. The egg production, fertility, hatchability and shell thickness, gross and micropathology, and the development of porphyria were examined. Decreased hatchability of fertile eggs and neurological signs and death ensued at 80 mg/kg in the diet. Porphyria and histopathological liver changes were found in quails fed 80 and 5 mg/kg diet. A concentration of 1 mg/kg diet fed to quail did not show any adverse effects, and a NOAEL of 0.06 mg/kg b.w. per day can be derived from this study.

Adult male and female Japanese quail were fed HCB (purity >99 %) at 20 mg/kg diet (approximately 1.2 mg/kg b.w. per day) for 90 days to study the effects on their reproduction (Schwetz *et al.,* 1974). A decreased survival rate in the chicks hatched during the study was observed at the end of the study as well as increased liver weight in adult birds.

5.5. Rabbits

The oral LD_{50} for HCB in rabbit is above 2600 mg/kg b.w. per day (Strik, 1986).

New Zealand rabbits were mated and then administered HCB (purity >99.5 %) orally from days 1 to 27 of gestation at doses 0, 0.1, 1.0 or 10 mg/kg b.w. per day The purpose was to study placental transfer and foetotoxic effects (Villeneuve *et al.,* 1974). The dams were killed at day 28. No foetotoxic effects were observed at any dose.

5.6. Mink

Adult female and male mink were fed HCB at 0, 1 or 5 mg/kg diet from before mating and for the females feeding was continued during gestation and lactation, for a total of five months (Rush *et al.*, 1983). HCB had a profound effect on the survival of kits to weaning as mortality increased from 8 % in control to 44 and 77 % in the 1 and 5 mg/kg groups, respectively. Effects on the surviving offspring were determined at 16 - 17 weeks of age: body weight, weights and electronmicroscopy of liver and kidney, renal function and hepatic mixed function oxidases. Hepatic cytochrome P-450 content and ethoxyresorufin-O-deethylase activity were significantly increased in the 5 mg/kg group. The lowest concentration (1 mg/kg diet) given to mink showed an adverse effect, and a LOAEL of 0.05 mg/kg b.w. per day can be derived from this study.

5.7. Pets

Beagle dogs of both sexes, 7 - 10 months of age, were fed HCB at 0, 1, 10, 100 or 1000 mg/dog per day (corresponding to approximately 0.1, 1, 10 and 100 mg/kg b.w. per day and concentrations of 4, 40, 400 and 4000 mg/kg diet, respectively) in gelatine capsules for 12

months (Gralla *et al.,* 1977). Clinical health, haematology, clinical chemistry, gross and histopathology, and fluorescence microscopy of liver were examined. Anorexia, weight loss and mortality occurred at the highest dose and also, to a lesser degree, at the next lower dose. A dose related neutrophilia appeared in the animals receiving the two high dosages. The most widespread pathological lesions were confined to the abdomen and included serositis, necrosis, fibrosis, and steatitis of the omentum. Nodular hyperplasia of gastric lymphoid tissue was found in all treated dogs including those at 1 mg/dog per day. No hepatic fluorescence was found at necropsy, indicating that the dogs did not show porphyria. The LOEL from the study was 0.1 mg/kg b.w. per day.

Adult female beagle dogs were fed HCB at 50 - 150 mg/kg b.w. per day (corresponding to approximately 2000 - 6000 mg/kg diet) in gelatine capsules for 21 days followed by a 14-days period of reduced feed intake to remobilize the HCB stored in the body (Sundlof *et al.,* 1981). Clinical health, weight, electroencephalogram (EEG), clinical chemistry and haematology, gross and histopathology were examined. HCB induced liver and hepatocyte enlargement and physiological changes in the central nervous system. Weight loss and haematological changes were equivocal with treatment stress and HCB probably contributing to the changes to varying degrees.

The oral LD_{50} for HCB in cats is 1700 mg/kg b.w. per day (Osweiler *et al.,* 1985). The cat is among the most susceptible species to acute HCB exposure.

To investigate the effects of feeding HCB on reproduction in cats, mature queens were fed HCB for 142 days during gestation and nursing (Hansen *et al.,* 1979c). HCB was either added to uncontaminated baked ground pork (mean HCB level 260 mg/kg) or was present as residue in baked ground pork derived from pigs fed HCB (mean HCB level 90 mg/kg). The pork constituted about 50 % of the total feed intake, so the average HCB levels in the total diet for the cats fed pork with added HCB and pork with HCB residues were 130 and 45 mg/kg, respectively, (corresponding to on average 8.7 and 3 mg/cat per day, or approximately 3 and 1 mg/kg b.w. per day, respectively). Clinical, haematological, gross and histopathological examinations and porphyrin measurement were carried out. The pork diet from HCB fed pigs did not produce any statistically significant effects compared to the control group. The pork spiked with HCB significantly increased the relative liver size and mortality in the suckling kittens. There was also a tendency towards weight loss for the queens, reduced litter size and weight, and increased susceptibility to infectious disease in both suckling kittens and queens. An average concentration of 45 mg HCB residues/kg total diet fed to cats did not show adverse effects, and a NOAEL of approximately 1 mg/kg b.w. per day can be derived from this study. However, the bioavailability for the cat of the HCB residues (including possible metabolites) present in the pork is not commented in the paper and not known.

6. Toxicokinetics and tissue disposition

6.1. Absorption

Several studies on metabolism carried out in laboratory animals demonstrated that absorption of HCB from the gastrointestinal tract is dependent on the solvent vehicle. When HCB is administered in an oil vehicle, approximately 80 % of the dose (between 5 and 180 mg/kg b.w. per day) is absorbed (Albro and Thomas, 1974; Koss and Koransky, 1975; Mehendale *et al.,* 1975; Ingebrigtsen *et al.,* 1981; Ingebrigtsen and Nafstad, 1983). The absorption is limited (2 - 20 %, depending on the dose) when HCB is administered as an aqueous suspension, or in a solid crystalline form (Iatropoulos, 1975; Koss and Koransky, 1975). Absorption occurs primarily through lymphatic channels, with only a minor portion being absorbed into the portal circulation (Iatropoulos, 1975).

No quantitative data specifically describing the absorption of HCB in domestic animals following oral exposure were found in the literature. However, the presence of HCB residues in tissues, eggs or milk of animals exposed through diet (WHO-IPCS, 1997) indicates that gastrointestinal absorption occurred to a significant extent. In rainbow trout, HCB absorption through the gut was estimated to be between 80 and 90 % of the dose, depending on the HCB content of diet (from 4 to 780 µg/kg feed) (Niimi and Cho, 1980).

Quantitative data on absorption of HCB by humans are scarce. By comparing intake and faecal excretion of HCB in a single breastfed infant, Abraham *et al.* (1994) estimated that absorption was greater than 99.7 % at one month of age and greater than 97 % at five months. Gastrointestinal absorption of polychlorinated organic pollutants including HCB was investigated by Schlummer *et al.* (1998) in seven individuals (four males and three females) aged 24 to 81 years with different contaminant body burdens, using a mass balance approach. The difference between the ingested and excreted amounts of HCB was defined as net absorption. HCB food concentration was 0.59 - 1.05 µg/kg dry weight. In the four young adults tested (one female and three males ranging from 24 to 36 years) the percentage absorbed ranged from 70 to 82 %, whereas it was 1 % in a 53 year old male volunteer and was negative for the two older volunteers, indicating that HCB was excreted to a greater extent than ingested, probably because of a higher previous exposure.

6.2. Distribution

Following absorption, HCB is rapidly and widely distributed in mammalian tissues. Due to its lipophilic nature, HCB is most likely to be distributed to adipose tissue or organs with high fat content (WHO-IPCS, 1997; ATSDR, 2002). In all species studied, the greatest concentrations appeared in adipose tissue. In rats receiving a single oral dose of ^{14}C-HCB (0.4 mg/kg b.w. per day), peak levels of radioactivity in the fat were found to be roughly 60 fold higher than in the blood and 30 fold higher than in the brain and liver (Ingebrigtsen and Nafstad, 1983). Similar results were obtained by Koss and Koransky (1975) with higher doses (20 - 180 mg/kg b.w. per day). Other lipid-rich tissues such as the skin and the bone marrow may also contain relatively high concentrations compared with lean tissues. HCB may be accumulated

in the adrenal cortex at concentrations approaching those in fat (Sundlof *et al.,* 1982, Foster *et al.,* 1993, 1995). Other tissues (e.g. liver, kidneys, lungs, heart, spleen and blood) generally contain lower amounts on wet weight basis. In animals, HCB is readily transferred from the pregnant mother to the foetus through the placenta. Based on the results obtained in rats given 100 µg HCB/kg diet during pregnancy, Nakashima *et al.* (1997) estimated the amount of HCB transferred from a dam to her foetuses to be about 0.4 % of her total intake during pregnancy.

As expected, the adipose tissue in dogs contained the greatest concentration of HCB. They were given a single daily oral dose of 10 or 100 mg of HCB per kg b.w. per day for seven consecutive days, and killed on the eighth day (Sundlof *et al.,* 1982). Other lipid reach tissues, such as the skin and adrenal glands, accumulated relatively high concentrations compared with lean tissues. In addition, the thyroid was found to have a relatively high affinity for HCB.

In four to five month old lambs exposed for 90 days to HCB at a dietary concentration of 0, 0.01, 0.1 and 1.0 mg/kg feed, the adipose tissue concentrations reached a level approximately ten times that in the diet at the end of the exposure period and the highest HCB concentrations in other tissues were found in the brain and liver (Mull *et al.,* 1978). In sheep dosed orally for 18 weeks with HCB at 0.1, 1.0, 10.0, and 100.0 mg/animal/day, corresponding to approximately 0.1, 1.0, 10.0, and 100.0 mg/kg feed, maximum concentrations of HCB were found in adipose tissue, equivalent to 7 - 9 times the concentration in the feed, independent of the doses studied (Avrahami and Steele, 1972a). In these species, the concentrations of HCB in blood are about 1000 times lower than the levels found in adipose tissue.

The disposition of HCB was studied in swine throughout gestation and nursing (Hansen *et al.*, 1979a). Animals were fed diets containing 1 or 20 mg HCB/kg feed. Because of differences in the oestrous cycles the duration of treatment before farrowing ranged from 141 to 170 days, whereas the total exposure period ranged from 183 to 232 days. At the end of the experiment the residues in the adipose tissue and bone marrow of the sows was five to seven fold higher than the dietary concentrations. The concentrations in the other tissues were in the following order: adrenal gland > brain > muscle, liver.

In male broiler chicks fed from one day old for eight weeks on a diet containing 0.6, 6.0, 30 and 120 µg HCB/kg feed (Reed *et al.,* 1977), HCB concentration was highest in adipose tissue, followed by the heart, gizzard, leg, kidney, liver, and breast. In laying hens treated for seven consecutive days with oral doses of HCH ranging from 1 to 100 mg/kg b.w. per day (corresponding to about 10 to 1000 mg/kg feed), the concentration in the main tissues was as follows: adipose tissue > skin > liver > heart > brain > blood > muscle (Hansen *et al.,* 1978).

The distribution of HCB in green sunfish (*Lepomis cyanellus*) fed ^{14}C-HCB at 1, 10 and 100 mg/kg feed for three consecutive days (Sanborn *et al.,* 1977) was investigated. Throughout the course of the experiment (days 4, 14 and 28), the highest residues were in the alimentary tract of the fish (stomach, pyloric caeca, intestine), the next highest residues were found in the liver and the remaining carcass, and the lowest residues were found in the skeletal muscle.

Several authors have found HCB in human milk, blood plasma and adipose tissue (mainly from breast) (ATSDR, 2002). For recent values in human milk see chapter 8.

6.3. Metabolism

In mammals, HCB metabolizes slowly into other less chlorinated benzenes, chlorinated phenols, and other minor metabolites. Koss *et al*. (1986) identified 21 different metabolites in the urine of rats exposed to HCB. Three distinct pathways appear to operate in mammals (Figure 3): oxidation which gives rise to phenolic metabolites including pentachlorophenol, tetrachlorohydroquinone and tetrachlorobenzoquinone; glutathione-conjugation with loss of chlorine leading to corresponding cysteine conjugate, then to mercapturic acids, and finally to pentachlorobenzenethiol, pentachlorothioanisole and other sulfur-containing metabolites; a minor reductive dechlorination pathway that yields less chlorinated benzenes (Renner, 1988; WHO-IPCS, 1997).

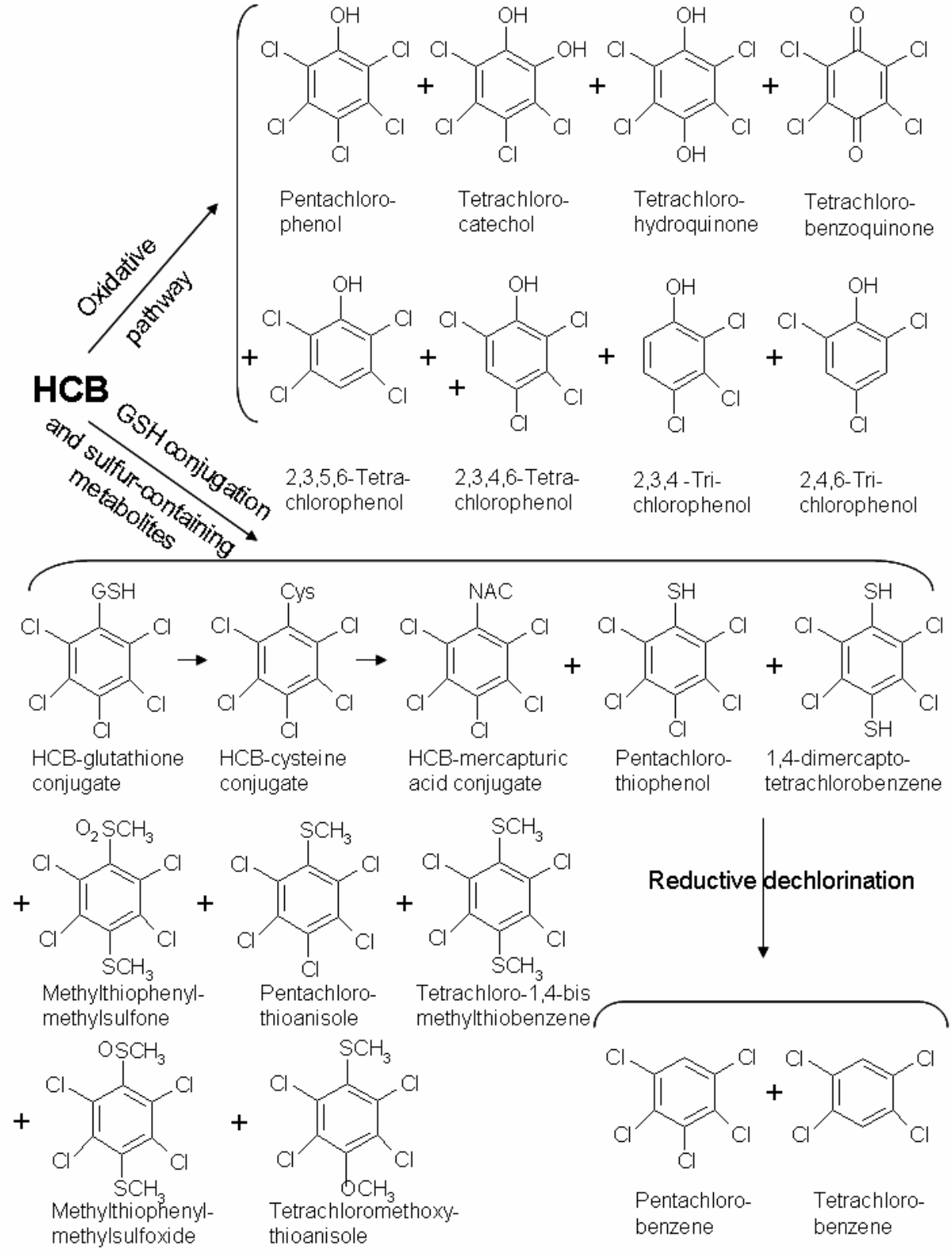

Figure 3. Major metabolic pathways of HCB in mammals.

The oxidative attack on HCB, mediated by cytochrome P-450 monooxygenases, results in the formation of an electrophilic intermediate, typically an epoxide that covalently interacts with tissue macromolecules (Van Ommen *et al.*, 1985, Rietjens *et al*., 1997). Alternative pathways

that compete for interaction with the epoxide, include spontaneous isomerization of the epoxide to a phenol, epoxide hydrolase-catalyzed conversion of the epoxide to a dihydrodiol, and either spontaneous or glutathione-S-transferase-mediated conjugation of epoxides with glutathione. Alternatively, the phenol may undergo further oxidation to form the hydroquinone and quinone metabolites.

The monitoring for metabolic products of HCB included excretory products and/or tissue residues from rats, mice, guinea pigs and monkeys. Findings were quite dissimilar (qualitatively and quantitatively) among the studies.

Other studies in rodents identified pentachlorobenzene, pentachlorophenol, pentachlorothiophenol, tetrachlorohydroquinone, tetrachlorobenzene, tetrachlorobenzene-1,4-diol, and 2,4,5-trichlorophenol as major urinary metabolites following oral HCB exposure (Koss *et al.,* 1978; Mehendale *et al.,* 1975; Rizzardini and Smith, 1982; Rozman *et al.,* 1978).

In mammals, the faeces contain mostly unchanged parent compound, and about 1 % pentachlorobenzene and traces of pentachlorophenol after oral HCB exposure (WHO-IPCS, 1997).

Pentachlorothiophenol, pentachlorophenol, methylthiopentachlorobenzene, 1,4-bis-(methylthio)-2,3,5,6-tetrachlorobenzene, chlorophenols, S-conjugated phenols and chlorobenzenes, as well as less chlorinated benzenes have also been identified in the liver following oral HCB exposure in rat and mice (ATSDR, 2002; D'Amour and Charbonneau, 1992; Renner, 1988).

In the more recently published *in vivo* metabolic study (den Besten *et al.,* 1994), *N*-acetyl-*S*(pentachlorophenyl)cysteine was the most abundant urinary product in female Wistar rats with a dietary exposure to HCB for 13 weeks and resulting in doses of 7.5 or 15 mg/kg per day. Pentachlorophenol, tetrachlorohydroquinone and mercaptotetrachlorothioanisole, which was excreted as a glucuronide, was also detected in the rat urine samples.

In rats exposed through diet to HCB, the compounds found in adipose tissue were pentachlorobenzene, pentachlorobenzenethiol, bis(methylthio)-tetrachlorobenzene and pentachloroanisole in addition to the parent compound (WHO-IPCS, 1997).

Significant sex-related differences were observed, with higher amounts of pentachlorothiophenol observed in the livers of female rats. This was accompanied by a slower decrease in hepatic levels of HCB in the female rat liver compared to the male liver (Richter *et al.,* 1981). After 10 weeks of treatment of the rats with HCB, the urinary excretion of pentachlorophenols, 2,3,5,6-tetrachlorobenzene-1,4-diol, and pentachlorothiophenol was greater in females than in males (Rizzardini and Smith, 1982).

These sex-related differences in biotransformation of HCB could account for differences observed in the toxicological effects of HCB in male and female rats.

6.4. Excretion

Excretion of HCB and metabolites occurs slowly, through urinary and faecal routes, but faecal excretion is by far the most important route. During seven days following a single dose of ^{14}C-HCB to adult male rats (5 mg/kg b.w. per day, administered by oral intubation in arachis oil), approximately 16 % of the dose was excreted in the faeces and less than 1 % in urine (Mehendale *et al.,* 1975). In a study performed in rats dosed intragastrically with ^{14}C-HCB, Ingebrigtsen *et al.* (1981) showed that less than 4 % dose was recovered in the bile of bile-duct-cannulated animals. These data demonstrate that faecal excretion results mainly from unabsorbed HCB and only in a limited extent from biliary excretion. This finding was further supported by a study in rhesus monkeys in which complete biliary bypass did not alter HCB elimination via the faeces (Rozman *et al*., 1983; Rozman, 1985).

In beagle dogs receiving single daily oral doses of 10 or 100 mg of ^{14}C-HCB per kg for seven consecutive days (Sundlof *et al.,* 1982), cumulative urinary excretion represented < 6 % of the total ingested dose after 12 weeks, whereas faecal excretion accounted for 44 % of the dose during the same period. Compared to other species in which faecal excretion mainly represents unabsorbed HCB, faecal elimination of HCB in the dog is predominately due to metabolites excreted via the bile.

A number of studies demonstrate that a significant portion of the maternal HCB body burden may be eliminated through breast milk (ATSDR, 2002).

Elimination half-lives for HCB range from about one month in rats and rabbits, to two or three years in monkeys (WHO-IPCS, 1997). In beagle dogs, the half-life of HCB has been estimated to vary from 1.5 to 35 months (Sundlof *et al.,* 1982). The values reported for sheep, lambs, and pigs are approximately 10 - 18 weeks (Mull *et al.,* 1978; Avrahami, 1975; Avrahami and Steele, 1972a).

In fish, an estimated half-life of 8 - 20 days was proposed by Sanborn *et al.* (1977) for the green sunfish fed an HCB contaminated diet, however, this finding was based on only two points (14 and 28 days post exposure) and did not account for dilution of the residues due to the relative increase in body weight over the study period. Making the appropriate adjustment regarding growth dilution, the half-life was estimated to be at least seven months in rainbow trout (Niimi and Cho, 1981).

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 HCB to dairy cattle and laying hens. The transfer ratio of HCB calculated on a milk fat basis was between 2.0 - 10.5, and the transfer ratio determined for whole eggs was between 1.3 and 5.5. No data was found on the excretion of HCB metabolites into milk and eggs.

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 HCB in adipose tissue of different species have been reported. In a series of feeding experiments involving sheep, laying hens and growing chickens, Avrahami and Steele (1972a,b,c) demonstrated that feeding dietary levels of HCB ranging from 0.1 to 100 mg/kg feed resulted in rapid accumulation of HCB in omental fat (sheep) and body fat (chickens and laying hens). Sheep stored HCB in body fat to the extent of seven to nine times the feed level at all levels tested. For chickens and young hens, the accumulation ratios ranged from 30 to 20 with increasing feed concentration. In broiler chickens fed diets containing 0.6, 6.0, 30 and 120 µg HCB/kg feed (Reed *et al.*, 1977) for eight weeks, the levels found in adipose tissue were 11 to 18 times the concentration in the diet and a linear relationship was found between the dietary HCB level and its concentration in adipose tissue. Similar values were previously reported by Vos *et al.* (1972).

In pigs (mean weight 18 kg) fed diets containing 0.1, 1.0 and 10 mg HCB/kg feed for 16 weeks, residues in adipose tissue reached 0.68, 6.0 and 56 mg/kg respectively, indicating that at the end of the experiment concentrations in fat were six to seven times the concentration in the feed (Avrahami, 1975). In order to monitor accumulation rates, subcutaneous back fat was taken by biopsy before dosing started and at regular intervals thereafter. The steady state was reached in pigs on the lower dose rates, but not for those receiving the rations containing 10 mg/kg feed. In other experiments performed in pigs exposed to dietary levels of HCB ranging from 0.1 to 0.3 mg/kg feed (Verschuuren *et al*., 1973; Wit *et al.,* 1973), the accumulation ratio was estimated to range from 8 to 11 (as compared with 0.3 for lindane, 2 for dieldrin, 3 for alpha-HCH and 5 for DDT).

Bioconcentration of HCB from water to fish is well documented but little is known about the bioaccumulation occurring through dietary exposure. The transfer of HCB from fish feed to rainbow trout was investigated by Niimi and Cho (1980). Subadult trout (average mean weight 84 g) were fed a feed containing 4 (control), 394 and 780 µg/kg HCB for 57 days. The residues were monitored in whole fish (after removing residual materials from the intestine) sampled at day 20, 35 47 and 57 and the HCB intake was calculated on the basis of the amount of feed ingested over the four sampling intervals. Estimated transfer rates, calculated as percentage of HCB in whole fish in relation to the total doses administered via feed were 80 - 90 % for fish fed the HCB diets containing 394 and 780 µg/kg feed. The feeding of ^{14}C-HCB containing pellets (100 mg/kg feed) to green sunfish (*Lepomis cyanellus*) during three consecutive days resulted in residues consisting of HCB and pentachlorophenol (PCP) (Sanborn *et al.,* 1977). On day four, the ratio of PCP/HCB was 0.6, 0.5 and 1.1 in liver, muscle and carcass, respectively. However, the ratio increased in all tissues, except muscle, when dietary exposure to HCB ceased (experiment lasting until day 28), suggesting that in some cases PCP could be the major residue in fish exposed to HCB. These findings were not confirmed in other fish species (Kasokat *et al.,* 1989).

8. Human dietary exposure

8.1. Background human exposure levels

Human exposure to HCB is primarily from dietary intake of fatty foods of animal origin (Burton and Bennett, 1987; Schade and Heinzow, 1998; Sjödin *et al.*, 2000). Consequently, human HCB exposure is dependent on the type of food and the contamination level of the various food commodities. Jones (Eurochlor, 2005) gives a comprehensive summary on reported data concerning human background exposure, with special emphasis on various food consumption habits as well as occupational exposure to HCB.

Studies on recent dietary HCB intake are scarce. This is probably due to the long-standing ban on this compound as well as the progressive decline of HCB in humans as demonstrated by the analysis of human milk and serum samples over the course of time.

The dietary intake of some persistent organic pollutants was studied in 11 German women from the Land Schleswig-Holstein at the age of 22 - 40 years. A total of 55 duplicate samples were collected between April and May 2003. The sampling period for each participant was 5 days. The study revealed a HCB intake of 0.3 - 7.3 ng/kg b.w. per day. Mean, median and 95th percentile HCB intake was found to be 1.5, 1.1 and 3.5 ng/kg b.w. per day. Compared to 1997 where a similar study was conducted, the recent median dietary HCB intake is approximately 50 % lower (LGASH, 2005).

Dietary exposure assessment based on market basket studies was performed in the Czech Republic during the years 1994 to 2003 (Ruprich, 2003). A median dietary intake of HCB was estimated to be 2 ng/kg b.w. per day for this time period. The intake of highly exposed individuals, as represented by the 90th, 95th, 97.5th and 99th percentiles, were 3.8, 4.5, 5.1 and 5.9 ng/kg b.w. per day respectively. This recent median dietary intake is considerably lower than the corresponding value of 12 ng/kg b.w. per day estimated for 1994 (Ruprich, 1995).

Falco *et al.* performed a total diet study to assess the dietary HCB intake of the population of Catalonia in Spain between June and August 2000 (Falco *et al.*, 2004). Exposure estimates were based on the HCB concentrations in the food composites multiplied by the weight of the food group consumed by an average individual from Catalonia. The resulting total daily dietary HCB intake was found to be 6.4, 3.1, 2.5, 2.4 and 1.9 ng/kg b.w. per day for children (4 - 9 years), adolescents (10 - 19 years), male adults (20 - 65 years), female adults (20 - 65 years) and seniors (aged > 65 years). They also showed that the HCB intake by elite sportsmen and sportswomen is 94 % and 68 % higher than that for men and women of the general population. This is mainly due to the considerably higher intake of dairy products which represent around 77 % of the HCB intake by elite sportsmen and sportswomen (Falco *et al.*, 2005). Similar intake levels as for the general population in Catalonia were estimated in the 1990s for the Basque region in Spain (Urieta *et al.*, 1996), The Netherlands (Brussaard *et al.*, 1996) and Sweden (Vaz, 1995), reporting mean HCB intakes of 2.9, 1.4 - 3.1 and 5 ng/kg b.w. per day, respectively. The dietary HCB intake of 1.4 - 3.1 ng/kg b.w. per day estimated in The Netherlands in 1996 is considerably lower than the corresponding intake of 14.3 ng/kg b.w. per day reported in 1986 (Greve, 1986).

Somewhat lower dietary HCB intakes were derived from total diet analyses in the USA in the late 1980s and early 1990s. For 14 - 16 year-old males and 60 - 65 year-old women, the average intake was estimated as 1.1 and 0.6 ng/kg b.w. per day in 1988, 0.9 and 0.5 ng/kg b.w. per day in 1989, 0.5 and 0.2 ng/kg b.w. per day in 1990, and 0.4 and 0.2 ng/kg b.w. per day in 1991 respectively (ATSDR, 2002).

Dietary intake studies performed across Canada between 1993 and 1998 revealed an average daily human dietary HCB intake between 0.1 and 0.4 ng/kg b.w. per day (Health Canada, 2004).

The data indicate that the dietary HCB intake for adults and children is far below the health based guidance value of 170 ng/kg b.w. per day suggested by IPCS (IPCS, 1997).

In the framework of the 3rd WHO human milk field study (Malisch *et al.,* 2004), HCB 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 HCB levels ranged from 3 - 79 µg/kg milk fat with an overall median of 21 µg/kg milk fat. If only the European countries are considered, the HCB levels range from 12 - 79 µg/kg milk fat with a median value of 36 µg/kg milk fat. While the lowest levels were found in the specimens from Brazil and Fiji, the highest levels were determined in the samples from the Czech Republic, Spain and the Ukraine.

In an ongoing study since the late 1970s, human milk samples from women living in North Rhine-Westphalia/Germany were analysed for organochlorine pesticides. Figure 4 shows the levels of HCB in 1902 individual human milk samples collected and analysed between 1984 and 2001. It is clear that the ban and phase-out of HCB over the past two decades resulted in a substantial HCB decrease in human body burden and exposure to breast fed babies. Since 1984 the levels of HCB have decreased by almost 95 %. While the median level in 1984 amounted to 510 µg/kg milk fat, the respective median concentration in 2001 was only 30 µg/kg milk fat (Fürst, 2006).

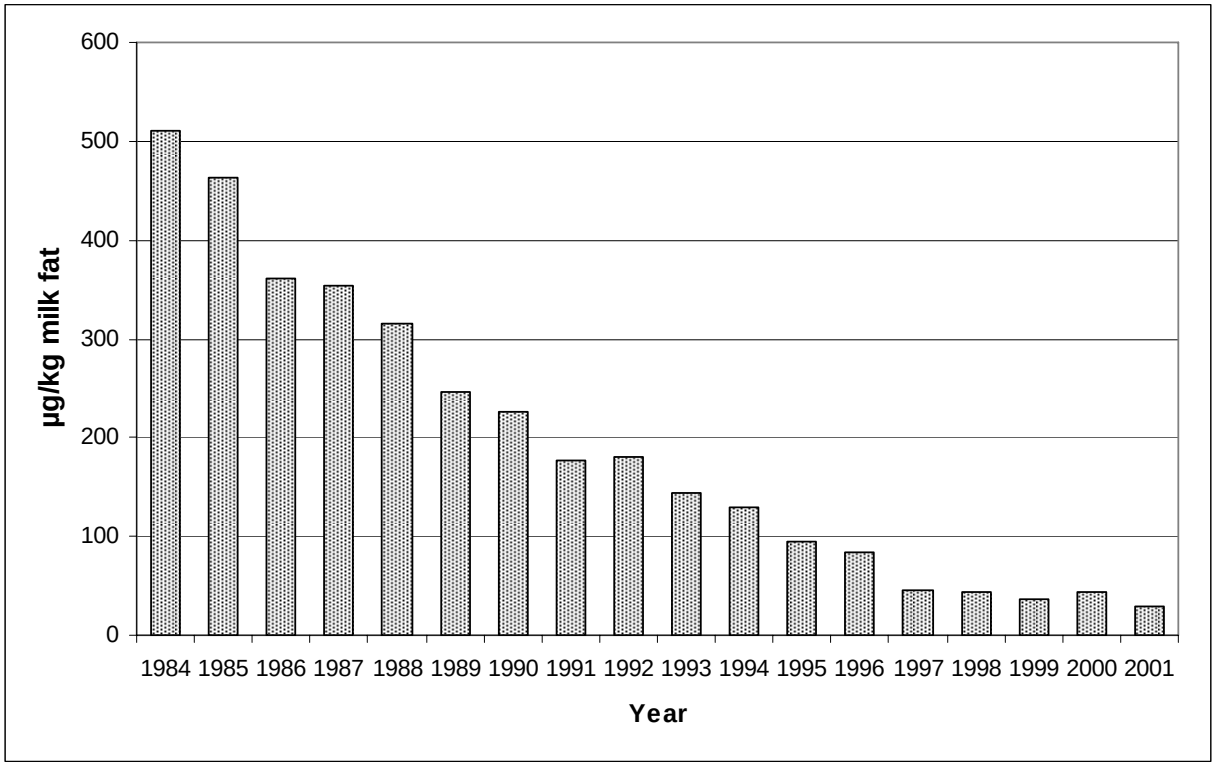

Figure 4. Median HCB levels in 1902 individual human milk samples from North Rhine-Westphalia/Germany in the course of time (Fürst, 2006).

Assuming an average daily intake of 800 ml breast milk with a fat content of 3.5 % for an exclusively breastfed baby weighing 5 kg, a HCB concentration of 36 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 200 ng/kg b.w. per day, which is approximately two orders of magnitude higher than the median daily intake in adults. Given its ongoing emission, its high persistency and its ability to bioaccumulate this justifies further measures to reduce human exposure to HCB.

CONCLUSIONS

Production, use and environmental fate

- HCB was used as a pesticide and still has some use as an industrial chemical. In addition, HCB is formed as a by-product in the manufacture of chlorinated compounds and also in thermal processes, such as incineration. Although the use of HCB as a pesticide was phased out in most countries many years ago, release of HCB into the environment still occurs to some extent and unusual high concentration in certain geographic areas.

- Because of the lipophilic properties and persistence in the environment, HCB is bioaccumulated and biomagnified along the food chain.

General toxicological effects

- The major target organ for HCB effects in experimental animals is the liver. Such effects include porphyria, and disturbances in metabolism of thyroid hormones. Other effects are immunotoxicity, reproductive toxicity and induction of tumours in the liver, kidney and endocrine organs. The mechanism of tumours induction in the kidney of male rats is not relevant for humans. With respect to thyroid tumours in rats and hamsters the mechanism of tumour induction is related to induction of hypothyroidism and compensatory hormonal stimulation of the gland, which is less relevant for humans. Although not definitive liver toxicity appears to be HCB induction of liver tumours. Despite these facts, HCB in some tests exhibited weak mutagenic activity and therefore a genotoxic mode of action could not be completely excluded. HCB has been classified by IARC as possible human carcinogen (group 2B).

Adverse effects of HCB in target animals

- HCB has a low acute toxicity to target animals.

- Oral exposure of **rainbow trout** to 0.78 mg HCB/kg feed in a study on toxicokinetics did not show any effect on body weight gains over 57 days. Effects were also not observed following an exposure to 7 mg/kg feed for 28 days (0.06 mg/kg b.w. per day). Higher feed concentrations have not been investigated. In short- or longer term experiments with fish exposed to HCB in water, no mortality or effects on growth were found after exposure to levels approaching its aqueous solubility (5 - 6 μg/L).

- Based on hepatic enzyme induction in a 90 day study in **lambs** a concentration of 0.1 mg/kg diet was identified to show no adverse effects, and hence a NOEL of 0.004 mg/kg b.w. per day can be derived.

- In a 13 weeks study in **growing pigs** a NOAEL of 0.05 mg/kg b.w. per day (corresponding to approximately 1 mg/kg feed) based on hepatic effects was derived.

- Japanese quail was the most sensitive **bird** species investigated. A concentration of 1 mg/kg in the diet did not show any adverse hepatic effects, and a NOAEL of 0.06 mg/kg b.w. per day can be derived. A concentration of 10 mg/kg diet fed to broiler cockerels did

not show any adverse hepatic and adrenal effects, and a NOAEL of 1 mg/kg b.w. per day can be derived. The lowest concentration (125 mg/kg diet) given to laying hens showed an adverse hepatic effect, and a LOAEL of 8 mg/kg b.w. per day can be derived.

- In **mink** the lowest concentration (1 mg/kg diet) showed an adverse effect on offspring mortality, and a LOAEL of 0.05 mg/kg b.w. per day can be derived.
- In **dogs** a LOAEL of 0.1 mg/kg b.w. per day (corresponding to approximately 4 mg/kg feed) in a one-year study was derived, based on hyperplasia of gastric lymphoid tissue.
- **Cats** fed HCB at approximately 3 mg/kg b.w. (corresponding to approximately 130 mg/kg diet) for 142 days during gestation and nursing showed increased liver size and mortality of the suckling kittens. Cats fed HCB residues (relay toxicity study) at approximately 1 mg/kg b.w. per day did not show statistically significant effects.

Contamination of feed and fate in animals including carry over

- Although the interpretation of the occurrence data of the various feed materials are hampered by the large differences in the respective limits of determination, the available data indicate that feed materials of animal origin, such as fish oil and fish meal are generally more contaminated than feed materials from plant origin.
- Depending on the contamination in soil, pumpkin seeds may contain elevated HCB levels due to their special ability for root uptake of HCB and its translocation into shoots. Whether other *Cucurbitaceae* have similar properties could not be determined.
- The concentrations determined in various feed categories including fish feed, generally indicate low levels (in the low μg/kg range) of HCB and thus are far below those that have been found to cause effects in fish and domestic animals. However, elevated levels may be found in feed materials that originate from polluted areas or from regions with recent HCB use.
- Absorption may vary from two to 80 % of the ingested dose, depending on species, doses and lipid content of the feed. HCB is metabolised slowly and is distributed to adipose tissue where it accumulates. The half-life varies from one month in rats and rabbits to two to three years in monkeys.
- In ruminants, transfer of HCB to milk is within the range of 2.0 to 10.5 % of the ingested dose. The transfer into eggs is between 1.3 and 5.5 % of the ingested dose. About 80 - 90 % of the amount of HCB ingested can be retained by fish. For pigs and chickens, the accumulation ratio was estimated to range from 8 - 11 and 11 - 30 respectively.

Effects on humans and human exposure

- At high HCB exposure major effects in humans are hepatic effects including disturbances of porphyrin metabolism. Other targets less frequently reported are the nervous system, skin, bone and thyroid gland.

- Data from total diet studies, as well as from human milk monitoring programmes performed in various EU Member States, show a considerable decline of up to 90 % in human HCB exposure over the past two decades.
- Food containing animal fat is the major source of HCB exposure in humans. Current studies indicate a mean dietary HCB exposure for adults and children (breastfed infants excluded) in the range of 0.1 to 5 ng/kg b.w. per day which is two to three orders of magnitude below the suggested health based guidance value of 170 ng/kg b.w. per day. The margin between the dose causing a 5 % increase above background of liver tumours in rats (0.81 mg/kg b.w.) and the human exposure range as given above is $1.6 – 80 \times 10^5$, which would indicate low concern from a public health point of view.

DATA NEEDS AND RECOMMENDATIONS

- Only few data exist on oral toxicity in fish. However, taking into account the long standing ban and the relatively low levels identified in fish feed there does not seem to be an urgent need for oral toxicity studies in fish.
- 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.
- Given the large variation of HCB levels in butter (and also human milk) samples and comparative high levels in certain regions, it seems appropriate to intensify the control of feed materials coming from these regions.
- The relevance of the uptake of HCB from soil in *Cucurbitaceae* for exposure via feed and food is not known. Hence, it seems appropriate to investigate whether the elevated levels in pumpkin seeds observed in certain areas also can be found in other regions. It would be also of relevance to explore whether this applies also to other *Cucurbitaceae* species.

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