OPINION OF THE SCIENTIFIC PANEL ON CONTAMINANTS IN THE FOOD CHAIN
ON A REQUEST FROM THE COMMISSION RELATED TO GAMMA-HCH AND OTHER
HEXACHLOROCYCLOHEXANES AS UNDESIRABLE SUBSTANCES IN ANIMAL FEED

Question N° EFSA-Q-2003-067

Adopted on 4 July 2005

SUMMARY

Technical hexachlorocyclohexane (HCH) is a mixture of various HCH isomers; alpha (α), beta (β), delta (δ) and gamma (γ) (also known as lindane). Both technical HCH and γ-HCH have been globally used as insecticides, and γ-HCH also for medical treatment in humans and animals. The insecticidal activity can be almost exclusively attributed to the γ-isomer. In some areas in the world these compounds are still in use. Because of the lipophilic properties and persistence in the environment, β-HCH followed by α-HCH and to a less extent γ-HCH may give rise to bioaccumulation and biomagnification through the food chain.

HCHs are rapidly absorbed from the gastrointestinal tract, pass the placenta and are transferred into milk. The toxicity of the isomers varies, γ-HCH being the most acutely neurotoxic followed by α-HCH. β-HCH penetrates less readily into the central nervous system, is more persistent and tends to accumulate in the body over time. All isomers cause liver hyperplasia and/or liver tumours. Except for experimental animals there are relatively few data on toxicity in other animal species. Neurotoxicity and liver effects have been reported in fish and ruminants. There is a lack of data on dose-effect relationship, particularly for fish and ruminants. β-HCH has weak estrogenic activity. α- and β-HCH are tumour promoters in rat liver. HCHs were classified by IARC in group 2B (possibly carcinogenic) on the basis of inadequate evidence for carcinogenicity to humans, sufficient (for technical grade and the alpha-isomer) and limited evidence for carcinogenicity to animals (for β- and γ-HCHs).

Data on occurrence in various feed categories including fish feed indicate levels in the μg/kg range of α-, β- and γ-HCH. Accumulation data in fish exposed through feed is lacking. The contamination route of HCHs into feed is not clear. However, the global trade of feedingstuffs and feed ingredients from regions with ongoing or recent use of HCHs may be a major source. A European reporting system, allowing for exposure assessment of undesirable substances in feed is missing.

Recent assessments of human dietary exposure to HCHs in Europe are scarce. Considering the available intake data from Czech Republic, Canada and USA and taking into account the decreasing concentration of HCHs in breast milk (about 80 % since the eighties in Germany),
current exposure through food is likely to be very low. $\beta$-HCH is usually still present but $\alpha$- and $\gamma$-HCH are only occasionally found in human milk samples from European Countries, which banned the production and use of technical HCH in the late 1970s. Some East European and developing countries with a longer use of technical HCH show higher contamination levels in breast milk.

**Key words:** HCHs, lindane, analysis, toxicity, residues in feed and food, carry-over, ADI, environmental fate.
TABLE OF CONTENTS

SUMMARY ................................................................................................................................. 1
TABLE OF CONTENTS .................................................................................................................. 3
LIST OF ABBREVIATIONS .......................................................................................................... 4
BACKGROUND ............................................................................................................................. 5
1. General Background ................................................................................................................ 5
2. Specific Background ............................................................................................................... 6
TERMS OF REFERENCE ............................................................................................................ 10
ASSESSMENT ............................................................................................................................... 10
1. Introduction ............................................................................................................................ 10
   1.1. Synthesis and chemistry ............................................................................................... 10
   1.2. Use and environmental fate ........................................................................................ 11
   1.3. Toxicology in laboratory animals and hazard assessment for humans ..................... 12
      1.3.1. Gamma-HCH ....................................................................................................... 13
      1.3.2. Alpha-HCH ........................................................................................................ 14
      1.3.3. Beta-HCH ......................................................................................................... 14
      1.3.4. Evaluations by other bodies .............................................................................. 15
2. Methods of analysis ............................................................................................................... 16
3. Statutory limits ..................................................................................................................... 16
4. Occurrence in feed and animal exposure .......................................................................... 17
5. Adverse effects on fish, livestock and pets, and exposure-response relationship .......... 18
   5.1. Introduction .................................................................................................................. 18
   5.2. Fish ............................................................................................................................. 18
   5.3. Ruminants ................................................................................................................... 19
   5.4. Pigs ............................................................................................................................ 20
   5.5. Birds ........................................................................................................................... 20
   5.6. Rabbits ....................................................................................................................... 21
   5.7. Dogs ........................................................................................................................... 21
6. Toxicokinetics and tissue disposition ................................................................................ 22
   6.1. Absorption .................................................................................................................. 22
   6.2. Distribution ................................................................................................................ 22
   6.3. Metabolism ................................................................................................................ 23
   6.4. Excretion .................................................................................................................... 24
7. Carry over and tissue concentration ................................................................................... 24
   7.1. Excretion into milk ..................................................................................................... 24
   7.2. Tissue levels ............................................................................................................... 25
      7.2.1. Pigs .................................................................................................................... 25
      7.2.2. Birds ................................................................................................................ 25
      7.2.3. Fish ................................................................................................................... 26
   7.3. Bioaccumulation in humans and experimental animals .............................................. 26
8. Human dietary exposure ....................................................................................................... 26
CONCLUSIONS ........................................................................................................................ 29
RECOMMENDATIONS ............................................................................................................... 30
SCIENTIFIC PANEL MEMBERS ............................................................................................. 39
ACKNOWLEDGEMENT ............................................................................................................ 39
DOCUMENTATION PROVIDED TO EFSA ............................................................................. 39

http://www.efsa.eu.int
LIST OF ABBREVIATIONS

ADI  Acceptable daily intake
ATSDR  Agency for Toxic Substances and Disease Registry
b.w.  Body weight
CYP  Cytochrome P450
DDE  1,1-Dichloro-2,2-bis(4'-chlorophenyl)ethylene
DDT  Dichloro-diphenyl-trichloroethane
EC50  Level of effect = 50% of the maximum effect.
ECD  Electron capture detector
GC  Gas chromatography
EMRL  Extraneous (maximum) residue limits
HCH  Hexachlorocyclohexane
IARC  International Agency for Research on Cancer
IPCS  International Programme on Chemical Safety
JMPR  Joint FAO/WHO meeting on pesticide residues
LD50  Dose that causes 50 % death
ML  Maximum level
MRL  Maximum residue level
MS  Mass spectrometry
NOAEL  No observed adverse effect level
OSPAR  The Convention for the Protection of the Marine Environment of the North-East Atlantic
PCB  Polychlorinated biphenyls
RfD  Reference dose
SCAN  Scientific Committee on Animal Nutrition
TDI  Tolerable daily intake
BACKGROUND

1. General Background


The main modifications can be summarized as follows:

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

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

During the discussions in view of the adoption of Directive 2002/32/EC the Commission made the commitment to review the provisions laid down in Annex I on the basis of updated scientific risk assessments and taking into account the prohibition of any dilution of contaminated non-complying products intended for animal feed. The Commission has therefore requested the Scientific Committee on Animal Nutrition (SCAN) in March 2001 to provide these updated scientific risk assessments in order to enable the Commission to finalise this review as soon as possible (Question 121 on undesirable substances in feed)\(^3\).

The opinion on undesirable substances in feed, adopted by SCAN on 20 February 2003 and updated on 25 April 2003\(^4\) provides a comprehensive overview on the possible risks for animal and public health as the consequence of the presence of undesirable substances in animal feed.

---

\(^1\) OJ L140, 30.5.2002, p. 10
\(^2\) OJ L 115, 4.5.1999, p. 32
\(^3\) Summary record of the 135th SCAN Plenary meeting, Brussels, 21-22 March 2001, point 8 – New questions (http://europa.eu.int/comm/food/fs/sc/scan/out61_en.pdf)
It was nevertheless acknowledged by SCAN itself for several undesirable substances and by the Standing Committee on the Food Chain and Animal Health that additional detailed risk assessments are necessary to enable a complete review of the provisions in the Annex, including the establishment of maximum levels for undesirable substances currently not listed.

2. Specific Background

Hexachlorocyclohexanes (HCHs) are a group of manufactured chemicals that do not occur naturally in the environment. HCHs have eight chemical forms (called isomers). The four predominant are α-, β-, γ and δ-HCH. The compound with the highest pesticide activity of these is γ-HCH, more commonly known as lindane.

The use of HCHs, containing less than 99.0 % of the γ isomer, as a pesticide was banned in the EU by Council Directive 79/117/EEC of 21 December 1978\(^5\) which prohibited the placing on the market and use of plant protection products containing certain substances as from 1 January 1981.

γ-HCH is an organochlorine insecticide and fumigant which has been used to control a wide range of soil-dwelling and plant-eating insects on a wide variety of crops, in warehouses and as a seed treatment.


The reason for the withdrawal was that assessments made on the basis of the information submitted have not demonstrated that it may be expected that, under the proposed conditions of use, plant protection products containing γ-HCH do satisfy the requirements with regard to the safety of operators potentially exposed to γ-HCH and with regard to the fate and behavior of the substance in the environment and its possible impact on non-target organisms.

Current EU legislation on maximum residue levels (MRLs) for pesticides is derived from/based on four Council Directives

- Council Directive 76/895/EEC of 23 November 1976 relating to the fixing of maximum levels for pesticide residues in and on fruit and vegetables\(^7\)

\(^5\) OJ L 33, 8.2.1979, p. 36
\(^8\) OJ L 221, 7.8.1986, p. 37


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

As the consequence of the coming into force of Directive 1997/41/EC, the pesticide residue legislation applies also to animal feedingstuffs since 1 January 1999. However some problems have been observed in implementing the pesticide residue legislation. The following problems have already been identified:

- compound feed is composed of a relatively high number of ingredients, of which several are processed products (by-products). It is not obvious to know what MRL is applicable to such compound feed as it involves many calculations and uncertainties and “unknowns” (processing factors),

- pesticide residue legislation does not cover products of marine origin which are regularly used in animal feed (no direct application),

- pesticide residue legislation does not cover products typically for animal feed (no food use) such as pastures, roughages, forages, fish oil and fish meal.

Therefore it is appropriate to include in the list of undesirable substances maximum levels for some pesticides in particular those of relevance for animal health or for public health through carry over from feed to food of animal origin.

The α isomers, the β isomers and the γ isomers are listed in the Annex to Directive 2002/32/EC.

---

¹⁹ OJ L 221, 7.8.1986, p. 43
¹¹ OJ L 70, 16/03/2005, p. 1
¹² OJ L 184, 12/07/1997, p. 33
The provisions on the maximum levels for α isomers and β isomers, prohibited in the EU for use as pesticide since a long time, in the Annex to Directive 2002/32/EC are comparable to the provisions foreseen in the pesticide legislation. For comparison, the current provisions in the EU-pesticide residue legislation are mentioned in Table 1 for the α and β isomers.

<table>
<thead>
<tr>
<th>Directive 2002/32/EC</th>
<th>EU-Pesticide residue legislation</th>
</tr>
</thead>
<tbody>
<tr>
<td>ML relative to a feedingstuff with a moisture content of 12 %</td>
<td>MRL applicable to the product as marketed</td>
</tr>
<tr>
<td>Product</td>
<td>mg/kg</td>
</tr>
<tr>
<td>HCH - α isomers</td>
<td></td>
</tr>
<tr>
<td>Fats</td>
<td>0.2</td>
</tr>
<tr>
<td>Other feedingstuffs</td>
<td>0.02</td>
</tr>
<tr>
<td>HCH - β isomers</td>
<td></td>
</tr>
<tr>
<td>Feedingstuffs for dairy cattle</td>
<td>0.005</td>
</tr>
<tr>
<td>Other compound feedingstuffs</td>
<td>0.01</td>
</tr>
<tr>
<td>Fats</td>
<td>0.1</td>
</tr>
<tr>
<td>Other feed materials</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Table 1. Current provisions in the EU pesticide legislation for α and β isomers.

The maximum levels for the α and β isomers of HCH in Directive 2002/32/EC are quite comparable to those in the pesticide legislation.

This is not the case for the γ isomer of HCHs, as the maximum levels in the Annex to Directive 2002/32/EC still reflect the situation before the ban on use of γ-HCH while the maximum residue levels for γ-HCH in pesticide residue legislation have been modified by Commission Directive 2002/66/EC of 16 July 200213.

For comparison the current provisions in the EU-pesticide residue legislation are mentioned in Table 2 for the γ isomers.

---

Table 2. Current provisions in the EU pesticide legislation for $\gamma$-HCH.

Before the entry into force of Commission Directive 2002/66/EC the following MRLs for $\gamma$-HCH were applicable to fruits and vegetables: leaf vegetables – 2 mg/kg, tomatoes, stone fruit and grapes – 0.5 mg/kg carrots – 0.1 mg/kg and other fruits and vegetables - 1 mg/kg. No maximum levels were previously set at EU level for cereals and foodstuffs of animal origin.

The current maximum levels for poultry meat and for eggs are Codex MRLs, which are extraneous residue limits (EMRLs) These MRLs are not set at the level which would result from current use of plant protection products but take account of the fact that uses of the substance in the past have left residues which can be considered as contaminants.

It is important that these provisions concerning $\gamma$-HCH are also reviewed in the framework of Directive 2002/32/EC to take into account recent developments. Given the persistent nature of $\gamma$-HCH, its only recent prohibition at EU level, the continued use in other parts of the world and the fact that legislation on undesirable substances contrary to pesticide legislation does not take into account processing factors, it is not possible to simply extrapolate the provisions of pesticide residue legislation into the Annex of Directive 2002/32/EC. Therefore a risk assessment focused on the presence of $\gamma$-HCH in animal feed should be undertaken before legislation can be amended.

Also a risk assessment on the presence of the other HCH isomers, in particular the $\alpha$- and $\beta$ isomers, should be undertaken in order to enable the European Commission to assess the appropriateness of the current legal provisions as regard these isomers to protect the public and animal health.
TERMS OF REFERENCE
The European Commission requests the EFSA to provide a scientific opinion on the presence of hexachlorocyclohexane (HCH) isomers in animal feed.

This scientific opinion should comprise the

- determination of the toxic exposure levels (daily exposure) of HCHs 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 HCHs from the feed to the products of animal origin results in unacceptable levels of HCHs or of their 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 HCHs and the characterization, 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 HCHs
  - to the overall exposure of the different relevant animal species to HCHs,
  - 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

1.1. Synthesis and chemistry

Hexachlorocyclohexanes (HCHs) were first prepared by Michael Faraday in 1825 by adding chlorine to benzene in sunlight (Figure 1). The reaction product exhibits a strong musty odour and flavour (Anonymous, 1993).

\[
\text{苯} + 3 \text{Cl}_2 \xrightarrow{\text{Light}} \begin{array}{c}
\text{氯化环己烷}
\end{array}
\]

Figure 1: Synthesis of HCHs
HCHs are not planar structures like a benzene ring. Instead, they exist as chairs or boats arrangement, of which the chair is the most stable conformation. The manufacturing of technical grade hexachlorocyclohexane yields an isomeric mixture consisting of five major constituents. Depending on the orientation of the chlorine atoms, whether being axial (a) or equatorial (e), these isomers are named α-, β-, γ-, δ- and ε–HCH. The average composition of technical grade HCH as well as the conformation of the different isomers is given in Table 1 (Jürgen, 1996).

Table 3. Average composition and isomeric conformation of technical grade HCH.

<table>
<thead>
<tr>
<th>Isomer</th>
<th>Conformation</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>α-HCH</td>
<td>aaeccc</td>
<td>65 – 70</td>
</tr>
<tr>
<td>β-HCH</td>
<td>eeeccc</td>
<td>7 – 10</td>
</tr>
<tr>
<td>γ-HCH</td>
<td>aaeccc</td>
<td>14 – 15</td>
</tr>
<tr>
<td>δ-HCH</td>
<td>aeeccc</td>
<td>6 – 10</td>
</tr>
<tr>
<td>ε-HCH</td>
<td>aeeaeccc</td>
<td>1 – 2</td>
</tr>
</tbody>
</table>

1.2. Use and environmental fate

The insecticidal properties of HCH were described in the early 1940s. It was shown that these properties are due to the γ isomer only (Figure 2) while the other isomers, making up approximately 85 % of the technical mixture, are not active. Products that contain > 99 % γ–HCH are also named lindane after the chemist van der Linden who in 1912 discovered a separation method for the isomers from technical HCH mixtures.

Technical HCH contains a number of impurities, such as chlorinated benzenes, heptachlorocyclohexane and octachlorocyclohexane, which contribute to the unpleasant odour. Moreover, 2,3,7,8-tetrachloro-p-dioxin (2,3,7,8-TCDD) was found in technical HCH at a concentration of 13 µg/kg (Anonymous, 1993). In contrast, γ-HCH is a white or colourless, crystalline solid with an odorless to slight musty or aromatic odour. It is only slightly soluble in water, but fairly soluble in organic solvents. γ-HCH is relatively stable against acids, and oxidative and hydrolytic degradation. Under alkaline conditions a dehydrohalogenation occurs. HCHs belong to the group of organochlorine pesticides. Since the beginning of commercial production in the early 1950s, γ-HCH became one of the most widely utilised insecticides worldwide. It has been used as a spray for foliage, in soil applications, for seed treatment and in baits for rodent control. Furthermore, it has been applied on a variety of fruits, seed grains, vegetable crops, in forestry and for poultry and other livestock. Aside from agricultural applications, it has been used for wood and timber protection and also in human medicine for treatment of head lice.
\(\gamma\)-HCH was, and in a number of countries continues to be, commercially marketed under trade names, such as Agrocide, Ambrocide, Aparasin, Apathiria, Benesan, Benexane, BoreKil, BorerTox, Exagama, Gallogama, Gamaphex, Gammalin, Gamma-Col, Gamene, Gamiso, Gammex, Gammexane, Gamasan, Gexane, Isotox, Jacutin, Kwell, Lindafor, Lindaterra, Lindatox, Lorexane, New Kotol, Noviagam, Quellada, Steward, Streunex, Tri-6, Viton and others (Anonymous, 1993).

Figure 2. Chair conformation of \(\gamma\)-HCH

Besides \(\gamma\)-HCH, also technical grade HCH was applied in many countries as an insecticide. It is estimated that a total of 382,000 tonnes of technical grade HCH and 81,000 tonnes of \(\gamma\)-HCH were used in Europe from 1970 to 1996. This is equivalent to an estimated cumulative use of 259,000 tonnes \(\alpha\)-HCH, 20,000 tonnes \(\beta\)-HCH and 135,000 tonnes \(\gamma\)-HCH (Ospar, 2004). HCHs are volatile and when applied to field crops in particular, a relative high proportion of the isomers enters the atmosphere and is later deposited. The long-range atmospheric transport has led to a global distribution especially of the most stable isomers \(\alpha\)- and \(\beta\)-HCH. Their persistence and lipophilic properties results in a significant bioaccumulation along the food chain. \(\beta\)-HCH shows the highest persistence followed by \(\alpha\)-HCH. These isomers can be determined in biological samples even from areas where the use of technical HCH was banned many years ago. Moreover, the ratio of \(\alpha\)-, \(\beta\)- and \(\gamma\)-HCH in samples may give valuable hints whether and when technical HCH or \(\gamma\)-HCH has been applied.

1.3. Toxicology in laboratory animals and hazard assessment for humans

\(\gamma\)-HCH was evaluated by IPCS in 1991 (WHO-IPCS, 1991b) and by JMPR in 2002 (FAO/WHO, 2002). \(\alpha\)- and \(\beta\)-HCH were reviewed by IPCS in 1992 (WHO-IPCS, 1992). Most data exists for \(\alpha\)- and \(\beta\)-HCH, whereas there is very little information on \(\delta\)-HCH. The toxicity of the isomers varies. With respect to acute exposure, \(\gamma\)-HCH is the most toxic, followed by \(\alpha\)-, \(\delta\)-, and \(\beta\)-HCH. At chronic exposure, however, \(\beta\)-HCH is the most toxic followed by \(\alpha\)-, \(\gamma\)-, and \(\delta\)-HCH. The increased toxicity of \(\beta\)-HCH following chronic exposures is most likely due to its longer biological half-life and its accumulation over time in the body (ATSDR, 2003).
1.3.1. γ-HCH

γ-HCH induces several drug-metabolising enzymes including the CYP system, glutathione-S-transferase and UDP-glucuronosyl transferase, but inhibits others such as epoxide hydrolase (FAO/WHO, 2002).

Numerous cases of fatal human poisoning and non-fatal illness caused by γ-HCH have been reported (Hayes, 1982). Symptoms of γ-HCH intoxication are seizures, convulsions, vomiting and dizziness (Davies et al., 1983; Kurt et al., 1986; Petring et al., 1986; Berry et al., 1987). In epidemiological studies high levels of γ-HCH (three times that of the general population) were found in women who went into premature labour (Saxena et al., 1981; Wassermann et al., 1982). Medical surveillance of workers employed in γ-HCH production have generally not revealed toxic effects of any significance, except for possible minor changes in neurological state and in electroencephalograms, as observed in one study (Czeglédi-Janko and Avar, 1970).

Orally dosed γ-HCH is moderately neurotoxic in mice and rats with NOAELs of 6 - 7 mg/kg b.w./day in acute and sub-chronic studies (FAO/WHO, 2002). LD₅₀s were 88 - 190 and 59 - 562 mg/kg b.w./day in rats and mice, respectively (OSPAR, 2004).

In long- and short-term oral studies of toxicity and studies of reproductive toxicity in rats, γ-HCH was found to be toxic to kidney and liver. Renal toxicity of γ-HCH was specific to male rats and a consequence of accumulation of α₂micro-globulin, a protein that is not found in humans and this effect is therefore not of relevance for humans (FAO/WHO, 2002). Partially reversible hepatocellular hypertrophy was observed in a number of studies on rabbits, rats and mice. In a 2-year study of toxicity and carcinogenicity in rats, increased liver weight, hepatocellular hypertrophy and increased spleen weight were observed with a NOAEL of 10 mg/kg of diet (equal to 0.47 mg/kg b.w./day) (Amyes, 1990).

γ-HCH is not carcinogenic in rats or dogs, but increased incidences of adenomas and carcinomas of the liver were observed at a dose of 23 mg/kg b.w./day in a specific strain of mice, agouti and pseudoagouti mice; whereas other strains of mice did not show a clear tumourigenic response to γ-HCH (FAO/WHO, 2002).

γ-HCH at non-cytotoxic concentrations was not genotoxic in vivo or in vitro.

γ-HCH had anti-estrogenic properties in several studies in mice and rats with effects at doses of 5 mg/kg b.w./day or higher (FAO/WHO, 2002).

In developmental studies in rats, including a multi-generation study, reduced survival and decreased body weight were observed in addition to increased incidence of supernumerary ribs and delay in tooth eruption and hair growth (FAO/WHO, 2002). The critical effect in these studies was neurotoxicity with a NOAEL of 0.8 mg/kg b.w./day (Myers, 1999).

In its derivation of an ADI, JMPR considered the existing database adequate to also characterize the potential hazard of γ-HCH to foetuses, infants as well as children. They established an ADI of 0 – 0.005 mg/kg b.w. on the basis of the NOAEL of 0.47 mg/kg/
b.w./day, in the long-term study of toxicity and carcinogenicity in rats using a safety factor of 100. An acute RfD of 0.06 mg/kg b.w. was also established on the basis of the NOAEL of 6 mg/kg b.w./day in the study of acute neurotoxicity in rats, using a safety factor of 100 (FAO/WHO, 2002).

1.3.2. \( \alpha \)-HCH

Acute oral LD\(_{50}\) values for \( \alpha \)-HCH lie between 0.5 - 5 g/kg b.w. in rats and mice. Signs of intoxication were mainly from the nervous system.

In a 90-day study rats given 0, 2, 10, 50 or 250 mg \( \alpha \)-HCH/kg diet showed growth depression at the highest dose (equivalent to 12.5 mg \( \alpha \)-HCH/kg b.w./day). Liver hypertrophy was seen at a dose of 10 mg/kg diet (equivalent to 0.5 mg/kg b.w./day). The NOAEL was 2 mg \( \alpha \)-HCH/kg diet (equivalent to 0.1 mg/kg b.w./day). Signs of immunosuppression (reduced levels of immunoglobulines) were seen at 2.5 mg \( \alpha \)-HCH/kg b.w./day (Kuiper \textit{et al.}, 1985).

No adequate long-term toxicity studies or studies on reproduction and teratogenicity have been identified for \( \alpha \)-HCH.

Mutagenicity data for \( \alpha \)-HCH are limited. \( \alpha \)-HCH was not mutagenic in \textit{Salmonella typhimurium} strains TA98, TA100, TA1535, or TA1537 with or without metabolic activation (Lawlor and Haworth, 1979; Nishimura, 1982).

Studies on initiation-promotion show that \( \alpha \)-HCH is a tumour promoter in the liver of mice and rats (WHO-IPCS, 1992).

Several long-term studies (> 24 and up to 107 weeks) have been carried out in rodents. In rats and mice given 100 to 600 mg \( \alpha \)-HCH/kg diet hyperplastic nodules and/or hepatocellular adenomas were found in mice only. Two mice studies and one rat study, using dose levels of up to 160 mg/kg diet (mice) and 640 mg/kg diet (rats) did not show any increase in the incidence of tumours. The absence of mutagenic activity in \textit{in vitro} studies indicates that the \( \alpha \)-HCH-induced tumourigenicity observed in mice has a non-genetic mechanism (WHO-IPCS, 1992).

The major urinary metabolite of \( \alpha \)-HCH in rats, 2,4,6-trichlorophenol, was reported by IARC (1987) to be carcinogenic for animals (classified as a chlorophenol in group 2B).

1.3.3. \( \beta \)-HCH

Oral LD\(_{50}\)s for \( \beta \)-HCH were 8 and 16 g/kg b.w./day for rats and mice, respectively. Signs of intoxication were mainly from the nervous system. However, \( \beta \)-HCH penetrates the blood brain barrier less readily than the other isomers.

In a 90-day study in rats given \( \beta \)-HCH liver changes similar to those induced by \( \alpha \)-HCH were seen at 2.5 mg \( \beta \)-HCH/kg b.w./day. Some gonadal effects were also seen at doses of 7.5 and 12.5 mg/kg b.w./day. The NOAEL was 2 mg \( \beta \)-HCH/kg diet (equivalent to 0.1 mg \( \beta \)-HCH/kg b.w./day) (van Velsen \textit{et al.}, 1986).
In a long-term rat study reported in 1950 doses of 10, 100 or 800 mg β-HCH/kg diet (equivalent to 0.5, 5 and 40 mg/kg b.w./day, respectively) all led to liver enlargement and histological changes (Fitzhugh et al., 1950).

In a two-generation reproduction study on rats exposed to β-HCH liver changes were found. A dose level of 10 mg/kg diet resulted in increased mortality and infertility. The NOAEL was 2 mg β-HCH/kg diet (equivalent to 0.1 mg β-HCH/kg b.w./day). No compound-related teratogenic effects were found in an extension to this study (van Velsen et al., 1986).

Mutagenicity data for β-HCH are limited. β-HCH was not mutagenic in Salmonella typhimurium strains TA98, TA100, TA1535, or TA1537 with or without metabolic activation (Lawlor and Haworth, 1979; Nishimura, 1982). β-HCH was positive in an in vivo bone marrow metaphase test in rats (Shimazu et al., 1976; IARC, 1979).

A weak estrogenic effect has been described for β-HCH with the uterus as a target organ. The mechanism and significance of this effect are uncertain. β-HCH did not displace 17β-estradiol from its receptor (WHO-IPCS, 1992), but seems to indirectly activate the estrogen receptor, possibly via c-ErbB2 activation. β-HCH promotes transformation and invasiveness of MCF-7 human breast cancer cells (Zou and Matsumura, 2003). β-HCH was tested alone and together with o,p’-DDT, p,p’DDT and p,p’-DDE for estrogenic activity in MCF-7 cells (Payne et al., 2001). The combined effect was described as additive. The no-effect concentration for β-HCH was 37 nM (10.8 μg/L) in the cell culture medium.

The carcinogenic potential of β-HCH has been investigated in two studies on mice. In one study, 200 mg β-HCH/kg diet (equivalent to 40 mg/kg b.w./day) was given for 110 weeks, and liver enlargement, hyperplastic changes, and an increase in benign and malignant tumours were reported. In the other study, where 500 mg β-HCH/kg diet were administered for 24 weeks, no tumours were observed. In several initiation promotion studies in rats β-HCH was shown to be a tumour promoter in rat liver (WHO-IPCS, 1992).

A possible link between human exposure to HCH and breast cancer has been examined in several epidemiological studies. Most of them were equivocal or had very limited power to assess this hypothesis. A non-significant trend (P = 0.24) between β-HCH in serum and breast cancer risk was observed during a 17-year follow-up of a Copenhagen cohort (Høyer et al., 1998). Cross sectional studies have not supported this finding (Calle et al., 2002).

1.3.4. Evaluations by other bodies

The International Agency for Research on Cancer evaluated technical grade hexachlorocyclohexane and γ-HCH in 1979 (IARC, 1979) and the individual hexachlorocyclohexanes in 1987 (IARC, 1987) and concluded that for the technical grade and the α isomer there is sufficient evidence for carcinogenicity to animals, whereas this evidence is limited for the β and γ isomers. There is inadequate evidence for their carcinogenicity to human beings. The hexachlorocyclohexanes were classified in group 2B.

In 1992 Health Canada set a group TDI for all HCH isomers of 0.3 μg/kg b.w. (Feeley, 2005).
2. Methods of analysis

Prerequisites for a reliable analytical determination of γ-HCH and other HCH isomers are an exhaustive extraction combined with a meticulous clean up, efficient separation and a sensitive detection. A number of well-proven and validated methods for analysis of HCH isomers in various environmental and biological matrices are available. Currently, high-resolution gas chromatography with electron capture detection (GC/ECD) is the analytical method of choice not only to differentiate between the different isomers but also to separate them from possible interfering co-extractants. An efficient separation of HCH isomers from other interfering compounds, such as other organochlorine pesticides and polychlorinated biphenyls (PCBs) is especially important when using GC/ECD. The use of gas chromatographic separation on two capillary columns of different polarity in routine monitoring programmes is therefore necessary. Potential coelution problems can also be overcome by applying combined capillary gas chromatography/mass spectrometry (GC/MS) as an alternative method. Although being more selective, GC/MS methods do not necessarily offer a higher sensitivity compared to GC/ECD. In either case, care has to be taken during extraction and clean up of samples in order to avoid losses due to the volatility of the HCH isomers.

3. Statutory limits

The use of HCH as a pesticide, containing less than 99 % γ-HCH was banned in the EU by Council Directive 79/117/EEC of 21 December 197814 which prohibited marketing and use of plant protection products containing certain substances as from 1 January 1981.

The authorisation of γ-HCH for use as a pesticide was withdrawn at EU level by Commission Decision 2000/801/EC of 20 December 2000 as γ-HCH is not included in Annex I to Council Directive 91/414/EEC and the authorisations for plant protection products containing this active substance15 were withdrawn. The use of plant protection products, containing γ-HCH as an active substance, has been banned in the EU since 21 June 2002.


See also specific background.

14 OJ L 33, 8.2.1979, p. 36
16 OJ L140, 30.5.2002, p. 10
17 OJ L 115, 4.5.1999, p. 32
4. Occurrence in feed and animal exposure

Thousands of feed samples are analysed annually in the Member States within the frame of official feed control, with the aim to check compliance with legal limits. As the Commission only requires the Member States to report their results as compliant and non-compliant, these condensed summaries give almost no information on actual levels in feed. Furthermore, it is often not specified which compounds are covered by the analytical method applied nor are the limits of detection reported. When comparing the summary reports it is often difficult to differentiate between numbers of individual analyses on the one hand and number of samples on the other hand. Concentration levels for individual substances analysed rather than condensed summaries for compound groups would be essential for a better understanding of the occurrence situation of undesirable substances in different feed materials and compound feeds as a prerequisite for a meaningful risk assessment and finally for a derivation of a possible temporal trend of the respective compounds in the feed chain.

Analysis of 104 feeding stuffs of plant origin performed in 2003/2004 as part of official feed control in Germany revealed no positive results for α-, β- and γ-HCH. In all samples which included crops, maize, oil seeds, tubers roots, mineral feed and compound feed for ruminants, pigs, poultry, horses and pets the three HCH isomers could not be detected at a limit of detection of 0.001 mg/kg. In 2002 a total of approximately 1700 feed samples was analysed for α-, β- and γ-HCH in Germany. Only in two samples the maximum residue levels for α- and β-HCH were exceeded. About 800 feed samples were analysed in the Netherlands in 2002/2003 with no non-compliant samples reported. In Denmark 800 feed samples were analysed between 1998 and 2004 for a number of pesticides. Twenty-two samples contained γ-HCH at levels between 0.001 and 0.020 mg/kg feed (based on 12 % moisture content). In addition, 9 samples contained α-HCH at levels between 0.002 and 0.039 mg/kg feed (based on 12 % moisture content) and 3 samples contained β-HCH at levels between 0.006 and 0.009 mg/kg feed (based on 12 % moisture content). The highest levels were found in complete and complementary feedingstuffs for cattle and sows. The finding of α-HCH as the predominant isomer in some positive samples indicates that these feedingstuff contain material that has been treated with technical HCH rather than γ-HCH. The sources of HCH in the feedingstuff are unknown. Between 2000 and 2004 a total of 870 single and compound feeding stuffs were analysed for a number of organochlorine pesticides in Belgium. α-HCH could be determined in 5 samples with levels between 0.001 and 0.017 mg/kg feed (based on 12 % moisture content). Five samples contained β-HCH with levels ranging from 0.001 to 0.008 mg/kg and one sample contained γ-HCH at a level of 0.012 mg/kg feed, all based on a moisture content of 12 %. In all other feed samples HCH isomers could not be detected at a reported limit of quantification between 0.002 and 0.005 mg/kg product.

Sixteen fish meal samples were analysed in the Czech Republic in 2004 for α-, β-, γ- and δ-HCH and other undesirable substances. α-HCH could be detected in two samples at levels of 0.5 and 0.8 µg/kg. Eight samples contained γ-HCH with levels ranging from 0.5 – 1.0 µg/kg. In all other fish meal samples α-, β-, γ- and δ-HCH could not be detected at a limit of quantification of 0.5 µg/kg. Ten fish meal samples and 5 meat- and bone meal samples were
analysed in 2003 in the Czech Republic and showed no levels for α-, β-, γ- and δ-HCH above the limit of determination of 2 µg/kg. Analyses of 9 fish feed samples meant for carnivorous fish provided by European Feed Manufacturers' Federation showed levels of 0.2 – 1.1 µg/kg for α-HCH, 0.4 – 0.7 µg/kg for β-HCH and 0.1 – 0.5 µg/kg for γ-HCH. These samples were analysed with a highly sensitive GC/MS method.

As the use of HCH has become continuously restricted worldwide, the frequency of detection and the levels detected in the environment, and in feed and food are generally low and will continue to further decrease. On the other hand, human milk data (see chapter 8) as well as butter samples which were collected world-wide (Kalantzi et al., 2001) indicate that specimens and products from areas with current use of HCHs are higher contaminated than specimens from areas where lindane and especially technical HCH was phased out earlier. Hence, it can not be excluded that feed materials originating from countries with ongoing use of lindane and technical HCH may contain elevated levels of certain HCH isomers.

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

5.1. Introduction

γ-HCH has an acute toxic effect in mammals, which is much greater than that of any of the other isomers. On the other hand, β-HCH is the isomer most strongly retained in the body fat, and may therefore cause chronic toxicity at low doses due to continuous accumulation. The persistent isomers, β-HCH in particular, may contaminate the soil and vegetable feed, and thereby cause animal poisoning and residues in animal products (Brüne, 1980; Kampe, 1980). Inhalation and percutaneous absorption are in addition to ingestion, possible routes of exposure to γ-HCH as the pesticide also has been applied in animal sprays and dips. γ-HCH contaminated feed and sprays and dips have caused animal poisoning (Humphreys, 1988).

Most available toxicity data concern γ-HCH. Only a few studies are available for α-HCH (fish) and β-HCH (fish and birds).

5.2. Fish

In a 3-months feeding study, rainbow trout (200 - 250 g) were given α-HCH in concentrations 10, 50, 250 and 1250 mg/kg diet. The fish were examined after 2, 4, 8 and 12 weeks. No effects were found on growth, microsomal liver enzymes, brain cholinesterase, serum alkaline phosphatase, and the histopathology of the brain, liver and kidney (Canton et al., 1975). Calculated from common feed intake of 0.2 – 2 % of their body weight, the highest α-HCH concentration in the feed corresponds to 2.5 - 25 mg/kg b.w.

No other reports have been found on oral toxicity in fish fed diets contaminated with HCH.

For γ-HCH in water most LC₅₀ (96 hours) values in several fish species studied are within a range of 20 - 90 µg/L, with a majority around 50 µg/L (WHO-IPCS, 1991). However, for brown trout a lower LC₅₀ value of 2 µg/L was reported (Macek and McAllister, 1970). γ-HCH has been shown to be more toxic at higher temperatures, but still within the range of LC₅₀.
values mentioned above (Macek et al., 1969). Wild populations of mosquito fish from areas that had previously been treated with γ-HCH tolerated considerably higher concentrations than unexposed fish, indicating that fish, or at least this species, can adapt to γ-HCH (Boyd and Ferguson, 1964; Culley and Ferguson, 1969). Results from long term studies of γ-HCH in bluegill, fathead minnow and brook trout indicate that water concentrations 5 – 10 times below those causing 50% mortality are well tolerated for long periods (up to 18 months) and the endpoints were effects on growth, mortality as well as reproduction (Macek et al., 1976).

The LC₅₀ of α-HCH in guppy exposed for 48 hours was 3.5 mg/L (WHO-IPCS, 1992).

The effect of α-HCH in freshwater on guppy (age 3 - 4 weeks) at concentrations 0.2, 0.8 and 2 mg/L for 50 days showed an EC₅₀ (mortality and immobilization) of 0.8 mg/L (Canton et al., 1975). In guppies exposed to α-HCH in saltwater for 2 - 4 days the EC₅₀ (mortality and immobilization) was 1.3 - 1.4 mg/L (Canton et al., 1978).

In guppy exposed to β-HCH for 48 hours the LC₅₀ was 0.9 mg/L (WHO-IPCS, 1992).

In a series of experiments on guppy and the Japanese ricefish medaka exposed to β-HCH through water (32 to 1000 µg/L), effects were studied after 1 - 3 months of exposure (Wester et al., 1985; Wester and Canton, 1986). The guppies were 3 - 4 weeks of age at start of the experiment. Histopathological changes indicating an estrogenic action were found at 100 µg/L and higher concentrations. The NOEC for induced formation of phosphoproteins (vitellogenin) was 32 µg/L. The β-HCH studies on medaka were started with freshly fertilized eggs or with 1 month old fish. The NOEC (behavioural effect; loss of buoyancy and balance, uncoordinated movements) was 56 µg/L when starting with fertilized eggs and 32 µg/L starting with 1 month old fish. Effect on growth, and histopathological lesions (indicating an estrogenic effect, and lesions in liver (vacuolation), kidney (glomerular hyalinosis) and thyroid gland (hypertrophy)) were found at higher concentrations.

5.3. Ruminants

In newborn calves and in older cattle and sheep the minimum lethal single dose of γ-HCH was approximately 5 mg/kg b.w. and approximately 25 mg/kg b.w., respectively (Radeleff et al., 1955). Two four-month old calves died within 1 hour or 50 hours, respectively, after ingesting approximately 29 or 13 mg/kg b.w. of γ-HCH (Frank and Braun, 1984). Cows accidentally fed 112 g of a powder containing 19.1 % of γ-HCH (45 mg γ-HCH/kg b.w.) died, and cows fed 70 g of the powder (28 mg γ-HCH/kg b.w.) survived with symptomatic treatment. These cows were pregnant at the time of dosing and were due to calve in six to 17 weeks. All calved at time with normal healthy calves (McParland et al., 1973). A single oral dose of 17 mg γ-HCH/kg b.w. administered to cattle produced convulsive attacks (Venturoli et al., 1973).

In sheep given γ-HCH orally at a concentration of 1.25 mg/kg in the feed daily for 165 days, suppressed cell-mediated immune response was reported. This was indicated by delayed type hypersensitivity reaction (Khurana et al., 1999).
5.4. Pigs

In pigs fed $\gamma$-HCH in the diet at 5, 10, 20, 40 and 80 mg/kg of feed over a period of nine months, no clinical, haematological or histopathological effects were observed (Schnell, 1965). The daily feed intake of weaned growing pigs is equivalent to approximately 4 – 6 % of their body weight (Pond et al., 1995). Thus, the highest diet concentration corresponded to approximately 3 - 5 mg/kg b.w./day.

No treatment-related effects were found on number of embryos, embryo weight or rate of ovulation in sows fed $\gamma$-HCH at 50 and 500 mg/kg diet from 30 days prior to mating until day 30 of gestation (Duee et al., 1975). The daily feed intake of non-lactating sows is equivalent to approximately 2 - 4 % of their body weight (Pond et al., 1995). Thus the highest diet concentration corresponded to daily doses of 10 - 20 mg/kg b.w.

5.5. Birds

The lethal dose of $\gamma$-HCH has been studied in various bird species, and covers a wide range. Most oral LD$_{50}$s of $\gamma$-HCH are in the order of 100 mg/kg b.w. (WHO-IPCS, 1991).

In white Leghorn x Australorp chickens fed 4, 16 and 64 mg $\gamma$-HCH/kg diet for 27 days increased mortality was found in the highest dosed group. In the two highest dosed groups a dose dependent liver hypertrophy was found (Harrison et al., 1963). The daily feed intake of Leghorn chicken is approximately 15 % relative to body weight after hatching, which is reduced to approximately 10 % after few days (Pond et al., 1995). Thus, the diet concentrations corresponded to approximately 0.4 - 0.6, 1.6 - 2.4 and 6.4 - 9.6 mg/kg b.w., respectively. The NOAEL in this study was 0.4 mg/kg b.w./day.

White Leghorn chickens and hybrid ducks were fed $\gamma$-HCH at 2, 4, and 10 mg/kg in the diet for three months and no adverse effects were observed in either species at any dose (Chen and Liang, 1956). The daily feed intake both in newly hatched Leghorn chicken and in ducklings is approximately 15 % relative to their body weight. The feed intake is within some days reduced to approximately 10 % for chickens and 7 % for ducklings (Pond et al., 1995). Thus, the highest dietary concentration corresponds to 1 - 1.5 mg/kg b.w. in the chicken and 0.7 - 1.5 mg/kg b.w. in the duck.

Laying hens fed diets containing $\gamma$-HCH up to 10 mg/kg for 60 days showed no effects on body weight gain, mortality, clinical symptoms or egg production (Ware and Naber, 1961). The daily feed intake of laying hens is approximately 6 % relatively to body weight (Pond et al., 1995). Thus, the highest diet concentration corresponds to 0.6 mg/kg b.w.

Whitehead et al. (1972) found that $\gamma$-HCH at 100 mg/kg of feed (corresponding to approximately 6 mg/kg b.w.) for one or two weeks had little effect on egg production of laying hens. Longer periods of feeding resulted in a decreased egg production, but did not significantly affect of egg shell thickness, egg and yolk weights and hatchability. The NOAEL was reported to be 10 mg/kg diet (corresponding to 0.6 mg/kg b.w./day).
In Japanese quail fed γ-HCH at 200 mg/kg for ten weeks, no significant effects on rate or pattern of egg production, egg size, shell thickness, calcium content, shearing strength or structure as seen through the scanning electron microscope (Whitehead et al., 1974). Expecting the daily feed intake at approximately 6% relative to body weight, the feed concentration corresponds to 12 mg/kg b.w.

Laying ducks were administrated γ-HCH by stomach tube at 20 mg/kg b.w. daily, three times a week or twice a week for eight weeks. Egg-laying stopped in the groups treated daily and three times weekly, but was less influenced in the group treated twice weekly (Chakravarty et al., 1986).

A flock of pigeons were fed a commercial whole grain pigeon feed contaminated with γ-HCH at 2100 mg/kg feed (Blakley, 1982). Soon after ingestion, diarrhoea, vomiting, anorexia and CNS depression were observed. The poisoning caused sudden death in 47% of the pigeon flock.

No information on oral toxicity of α-HCH was identified.

In chicken fed β-HCH up to 625 mg/kg diet for 12 weeks, no toxic effects on body weight, food consumption, growth, egg production, egg weight, shell quality, or mortality were observed. The highest concentration in the diet corresponds to approximately 38 mg/kg b.w./day (Kan et al., 1979; Pond et al., 1995).

5.6. Rabbits

The oral LD$_{50}$ of γ-HCH in rabbit is 200 mg/kg b.w. (Cameron, 1945).

γ-HCH was administered intragastrically to pregnant rabbits on days 6 - 18 of gestation at doses 5, 10, and 20 mg/kg b.w. All treated animals showed slight tachypnoea and lethargy during the treatment period and body weight gain and food intake were reduced. Post-implantation loss and the incidence of resorptions were increased at 5 and 20 mg/kg. The number of offspring with extra ribs (13th) was lower in animals given 5 mg/kg and higher in rabbits at 20 mg/kg than in controls. Foetal and litter weights were unaffected (Palmer et al., 1978).

5.7. Dogs

In dogs, a single oral dose of 40 mg γ-HCH/kg b.w. in oil was lethal. A dose of 30 mg produced convulsions, but was not lethal (Barke, 1950). In another study, 50 mg/kg of γ-HCH was lethal to four out of seven dogs (McNamara and Krop, 1948). In dogs (two males and two females) exposed to 15 mg/kg of γ-HCH in the diet (approximately 0.6 mg/kg b.w./day) for 63 weeks, no difference compared with controls was observed in mortality, body weight gain, organ weights, haematological parameters or histopathology (Lehman, 1965).

During a two-year toxicity study, groups of four male and four female beagle dogs were fed γ-HCH at concentration of 25, 50 or 100 mg/kg in the diet (equivalent to 0.8, 1.6 and 2.9 mg/kg
b.w./day). No treatment related changes were observed in feed or water consumption, body
weight, organ weights, or ophthalmology, electroencephalography (EEG), haematology,
clinical chemistry, urology, or histopathology, except increased alkaline phosphatase activity
and a darker and slightly enlarged liver were found in the highest dose group. The NOAEL in
this study was 1.6 mg/kg b.w./day (Rivett et al., 1978). In a supplementary group of dogs fed
γ-HCH at 200 mg/kg diet (equivalent to 6 mg/kg b.w./day) for 32 weeks, slight EEG-changes
were observed.

In beagle dogs given γ-HCH at 7.5 and 15 mg/kg b.w. daily during gestation, no significant
teratogenic effect or differences in number of living pups between control and test groups
were observed (Earl et al., 1973).

6. Toxicokinetics and tissue disposition

6.1. Absorption

Following oral administration, HCHs are rapidly and almost completely absorbed in rodents.
Sixty minutes after treatment, rats had absorbed more than 70 % of an intragastrically
administered dose of 1 mg/kg b.w. γ-HCH (Ahdaya et al., 1981). Albro and Thomas (1974)
estimated 95 - 99 % absorption of technical grade HCH within 4 days following a single oral
dose in rats. Variations of dose rates from 30 to 120 mg/kg b.w. had no influence on the
proportion absorbed. The overall absorption of technical grade HCH administered in the feed
for 14 days was similar, with average absorption values for α-, β-, γ- and δ-HCH of 97.4,
90.7, 99.4 and 91.9 %, respectively (Albro and Thomas, 1974).

Absorption of γ-HCH occurs to a significant extent in humans as demonstrated by high blood
concentrations of γ-HCH after incidences of accidental poisoning (Starr and Clifford, 1972;
Powell et al., 1980). In addition, several recent reports demonstrate the presence of residues
from HCHs (mainly β-isomer) in human blood plasma, probably resulting from consumption
of contaminated food (Sandanger et al., 2003, Walker et al., 2003).

6.2. Distribution

After oral administration to rodents, 14C-γ-HCH was rapidly and extensively distributed
throughout the body. The highest levels of radioactivity were detected in the fat of all
investigated species (Karapally et al., 1973; Chadwick et al., 1977; 1981; Chadwick and
Copeland, 1987).

In mice chronically fed γ-HCH, residues were found primarily in fat, and to a lesser extent in
brain, kidney, muscle, liver, adrenals and ovaries (Lahiri et al., 1990). A similar distribution
pattern was observed in rats (Khanna et al., 1995). In laboratory animals and in livestock, γ-
HCH has also been shown to cross the placenta and enter the foetus (Harrison and Mol, 1968;
Herbst and Bodenstein, 1972). β-HCH accumulates in most tissues to a greater extent than γ-
HCH. However, β-HCH is reported to cross the blood-brain barrier less readily than the other
isomers (Srinivasan and Radhakrishnamurty, 1983).
In rats fed 10 mg β-HCH/kg diet, the β-HCH level in adipose tissue was 60 mg/kg tissue and in the liver 45 mg/kg tissue after 56 days. The maximum concentration in the liver was reached after 4 weeks. During subsequent starvation, β-HCH was mobilized from adipose tissue by an enhanced lipid metabolism (WHO-IPCS, 1992). Rats exposed to β-HCH had a brain to blood ratio of 2:1 and a depot fat to blood ratio of about 170:1 over a range of blood concentrations (WHO-IPCS, 1992).

In pigs given diets containing γ-HCH at concentrations providing a dose of 0, 1 or 2 mg/kg b.w. for 21 days, the highest concentration was found in back fat; 0.19, 20 and 44 mg/kg were detected after treatment at 0, 1, and 2 mg/kg b.w./day, respectively (Davey and Gerrits, 1969).

In lactating goats given radio-labelled γ-HCH in doses of 1 - 10 mg/kg b.w. for 4 days the highest residue levels were detected in adipose tissue (approx. 2 % of the administered dose) followed by liver.

About 52 µg/g 14C-labelled residues was present in the fatty tissues of 1 year-old hen pheasants 1 day after they were fed 20 mg of 14C-γ-HCH as a single dose in gelatine capsules (Saha and Burrage, 1976). Mean 14C-labelled residues in muscle, brain and liver were 1.0, 2.5, and 3.1 µg/g, respectively. These levels decreased to 21.4, 0.6, 1.3, 0.1 in fat, muscle, brain and liver tissues 15 days later. About 78 and 64 % of the total residues were present as unmetabolised γ-HCH in fat and muscle, respectively, 1 day post-dosing. The proportion was 55 and 57 % respectively 15 days later, indicating further metabolism of γ-HCH.

After lethal acute intoxication of humans by HCHs, the HCHs concentration ratios, relative to that in blood, were 363:1 for fat, 3:1 for brain, and 15:1 for liver (WHO-IPCS, 1992).

6.3. Metabolism

Biotransformation involves dechlorination and dehydrochlorination followed by sulfo- and glucuronosyl conjugation. β-HCH appears to be more slowly metabolised than γ-HCH.

More than 70 metabolites of γ-HCH have been identified in animals and humans, and the biotransformation of γ-HCH appears to be mainly dependent on the CYP P-450 system. In rats, the major products found in urine were pentachlorophenol, 2,3,4,6- and 2,3,5,6-tetrachlorophenol and 2,4,6-trichlorophenol (Engst et al., 1976, 1979; Macholz and Kujawa, 1985). In the liver, 2,3,4,5,6-pentachlorobenzene and pentachlorocyclohexene were found in addition to the tetrachlorophenols. The kidney contains considerably higher levels of pentachlorocyclohexene than did the liver (Kujawa et al., 1977). Glutathione, glucuronide and sulfate conjugates of γ-HCH metabolites have been reported (Chadwick et al., 1978; Kurihara et al., 1979). Evidence exist that fish are able to metabolise γ-HCH to polar and non polar metabolites after exposure via surrounding water (Görge and Nagel, 1990) but no data were found for dietary exposure. In birds, metabolism of γ-HCH has been investigated in hen pheasants (Saha and Burrage, 1976) and in vitro with subcellular fraction from chicken liver (Foster and Saha, 1978). In both cases the major metabolites were pentachlorobenzene, isomers of di-, tri, tetra- and corresponding chlorophenols.
Although α-, β-, and γ isomers of HCH are biotransformed \textit{in vivo} at significantly different rates to produce a variety of excretable phenolic products, the β-isomer is much more slowly metabolized in rodents (Murphy, 1986). High residue levels of β-HCH in samples collected in biomonitoring studies give indirect evidence that this is also the case for other animal species and humans.

Human metabolism of HCHs was investigated by analysing the urine of workers potentially exposed to technical grade material (Engst \textit{et al.}, 1978). The analysis indicated the presence of α-, β-, γ-, and δ-HCH, traces of hexachlorobenzene and pentachlorobenzene, γ-, and δ-pentachlorocyclohexene, pentachlorophenol, 2,3,4,5-, 2,3,4,6- and 2,3,5,6-tetrachlorophenol, several trichlorophenols as well as glucuronides corresponding to these primary metabolites. In addition, pentachlorocyclohexanes, tetrachlorophenol, hexachlorobenzene and pentachlorobenzene were identified in blood samples. A similar metabolic pattern was reported by Angerer \textit{et al.} (1983) in the urine of workers producing γ-HCH. \textit{In vitro} investigations using human liver microsomes confirmed these metabolic pathways (Fitzloff \textit{et al.}, 1982) and demonstrated that an epoxide was formed during the metabolism of pentachlorocyclohexene (Fitzloff and Pan, 1984).

6.4. Excretion

The major route of excretion of HCHs and their metabolites is via urine, with a small portion of an oral dose eliminated in the faeces. The half-life of γ-HCH in rats was estimated to be 3 - 5 days, approximately 80 % of the administered dose being excreted within 8 days. The half-life for elimination from depot fat for γ-HCH is sex-dependent, i.e. 6.9 days in female rats and 1.6 days in male rats. Because of its high lipophilicity, γ-HCH can be excreted unchanged through the milk as demonstrated in laboratory animals (Dalsenter \textit{et al.}, 1997) and in humans (Nair \textit{et al.}, 1996, Waliszewski \textit{et al.}, 1999). Breast milk is a major route for the elimination of organochlorine pesticides in women. In human breast milk and adipose tissue the β-isomer is preponderant. Excretion into milk is also a significant elimination pathway in ruminants (see 7.1.)

In cows the half-life for elimination of β-HCH from fat was 4.2 - 22.0 weeks (Wolf, 1983).

In broilers the elimination half-life of β-HCH was about 6 - 8 weeks (WHO-IPCS, 1992).

Following cessation of continuous exposure the elimination of β-HCH in humans was slow with the concentration in fatty tissues decreasing only slightly over several years (Vohland and Koransky, 1983).

7. Carry over and tissue concentration

7.1. Excretion into milk

There are only limited data on carry-over from feed into milk.
Following a single HCH exposure of pregnant cattle the excretion in milk fat during the first 7 weeks after delivery was 0.7, 4.7 and 17 - 34 % for γ-HCH, α-HCH and β-HCH, respectively. Following long-term oral exposure of cows during lactation biological half-lives for excretion into milk were found to be 1, 2 - 3 and 3 - 5 weeks for γ-HCH, α-HCH and β-HCH, respectively. The corresponding carry-over rates for γ-HCH, α-HCH and β-HCH were 3, 9 and 30 – 37 % (Heeschen, 1985). However, no experimental details were given in this publication.

The γ-HCH concentration in the milk from lactating cows receiving diets containing lindane in gelatine capsules at doses ranging from 0.07 to 6.2 mg/kg b.w. for 70 - 180 days was measured daily. A dose-dependent increase was reported and the concentration ranged from 0.07 to 10 mg/L of milk (Ely et al., 1952).

In lactating Alpine goats given 14C-γ-HCH in gelatine capsules at a dose of 1 or 10 mg/kg b.w. for 4 days (Wilkes et al., 1987), the amount excreted in milk was 1 - 2 % of the administered dose and the concentration reached a plateau after 3 days. The major part of the radioactivity present in milk (85 %) was found in the fat.

7.2. Tissue levels

7.2.1. Pigs

Pigs of each sex received diets containing γ-HCH at concentrations providing a dose of 0, 2 or 40 mg/kg b.w./day (Davey and Johnson, 1974). Blood and back fat were collected at 6-week intervals for determination of residues. Whereas during most of the study the concentration in blood remained below 0.01 µg/mL, time- and dose-dependent increase in γ-HCH content was reported in the back fat samples.

7.2.2. Birds

Laying hen pheasants were administered a single dose 14C-γ-HCH (20 mg) in gelatine capsules or were fed with wheat seed containing 100 µg/g 14C-γ-HCH for 15 consecutive days (Saha and Burrage, 1976). In the latter group, the average total intake was 24.2 mg of 14C-γ-HCH per bird. Residues were determined in tissues of treated pheasants, in eggs, and in hatched chicks from both groups. Hen pheasants that had been fed 14C-γ-HCH treated seed had about 6.4, 0.2, 0.4, and 0.6 µg/g 14C-labelled residues in fat, muscle, brain, and liver, respectively, 1 day after starting of feeding. These levels increased to 28.8, 0.6, 1.7, and 1.9 µg/g, respectively, after 15 consecutive days of feeding. The levels of 14C-labelled residues in fat were about twice as high as those found in the egg yolk on the same day. Only traces of radioactivity were found in the albumen. Chicks contained significantly less 14C-labelled residues than the eggs from which they were hatched.

When low levels of HCHs were administered together with other organochlorine pesticides in the feed to broilers for 6 - 16 weeks, of the three HCH isomers tested (α, β, and γ), β-HCH showed the greatest bioaccumulation. The mean bioaccumulation factors for eggs and fat were 13 and 15, respectively (WHO-IPCS, 1992).
This relatively higher accumulation of β-HCH was also observed in chickens after feeding diets fortified with 1 mg β-HCH/kg for 4 weeks. The order of the degradation rate for the four HCH isomers was δ > γ > α > β. Biotransformation to one or more of the other HCH isomers did not occur (WHO-IPCS, 1992).

7.2.3. Fish

No data was found concerning the transfer of γ-HCH to fish via the feed chain. In an 18-day study in gudgeons placed in an aquarium supplied with water contaminated with γ-HCH (1 µg/L) and fed chironomidae larvae containing γ-HCH at a concentration of 50 µg/kg fresh weight, compared with gudgeons reared in the same contaminated water but receiving larvae contaminated at a 100 µg/kg level, no difference in γ-HCH level was found between the two groups (Marcelle and Thomé, 1984).

7.3. Bioaccumulation in humans and experimental animals

Based on the concentration of α-HCH in adipose tissue (0.03 and 0.02 mg/kg) of people from Germany and The Netherlands, and the corresponding concentration in the diet (1.3 and 0.3 µg/kg), the mean bioaccumulation factor (the ratio between the level in adipose tissue and the concentration in diet) of α-HCH for humans was calculated to 20.0 (range 11.5 - 32.5) (WHO-IPCS, 1992).

Similarly, based on concentrations of β-HCH in the human diets from several countries (0.68, 0.62, 1.0, 1.21, 0.56, and 0.67 µg/kg) and corresponding levels in adipose tissue (0.33 – 0.38, 0.40, 0.41, 0.9, 0.27 and 0.31 mg/kg, respectively), the mean bioaccumulation factor for β-HCH in humans was calculated to 527.0 (range 310 - 744) (WHO, 1992).

When β-HCH is administered repeatedly to rats, mice, and mini-pigs, there is marked storage in fat, especially in females, and the fat levels increase continuously as dosing progresses (WHO-IPCS, 1992). Data on concentrations in organs are contradictory: according to one report the levels in the kidneys, brain, and liver of rats reached a plateau after 4 weeks (Oshiba and Kawakita, 1972), whereas other sources reported steady increases in these organs throughout a 12-week dosing period (van Velsen et al., 1982).

8. Human dietary exposure

Food is the main source of exposure to HCHs for the general population. A number of total-diet and market basket studies were carried out in the 1970s and 1980s. In the USA, the average daily intake of α-HCH was 9 - 25 ng/kg b.w. during the period 1977 - 1979, and 3 - 16 ng/kg b.w. during the period 1982 - 1984. The average daily intake of β-HCH ranged from < 0.1 - 0.4 ng/kg b.w., for various age groups, in 1982 - 1984 (WHO-IPCS, 1991a). Total diet and market basket studies carried out in a number of countries led to estimates of the daily intake of γ-HCH up to 50 ng/kg body weight around 1970 and a gradual decrease to an intake of 3 ng/kg body weight per day or less in 1980. During a 4-years period in the mid-seventies, in the USA, the daily intake of γ-HCH by infants and toddlers decreased from 5 to 1 ng/kg body weight and from 10 to 5 ng/kg body weight, respectively (WHO-IPCS, 1991c). A total
diet study performed between 1993 and 1996 in Canada revealed average daily dietary intake levels for α-, β- and γ-HCH of 0.37, 0.39 and 1.32 ng/kg b.w., respectively (Health Canada, 2003). Recent representative dietary intake studies for European countries are scarce. Ongoing market basket studies performed between 1994 and 2003 in the Czech Republic, where HCHs were produced and used for a long time, indicate a decline of daily dietary intakes. While in 1994 the median daily intake for α-, β-, γ- and δ-HCH was 4.3, 8.4, 19.0 and 12.0 ng/kg b.w., respectively (Ruprich et al., 1995), the corresponding intake values in 2002 were reported as 1.6, 2.1, 6.4 and 4.4 ng/kg b.w., respectively (Ruprich et al., 2003). The Panel noted a somewhat higher concentration of δ-HCH relative to the other isomers in comparison with data from other countries. It cannot be excluded that this is due to a different composition of the technical HCH mixture produced in the Czech Republic; neither can coelution during the analytical determination be fully ruled out as a cause for this finding.

The continuous decline of exposure to HCHs is also substantiated by numerous investigations of human milk specimens from different areas of the world. Analyses of more than 2000 individual human milk samples from women living in Western Germany collected and analysed between 1984 and 2001 (Figure 3) indicate that the level of β-HCH has declined by more than 85 % during this period and currently amounts on average to about 0.020 mg/kg milk fat.

![Figure 3. Levels of α-, β- and γ-HCH in 2000 individual human milk samples from North Rhine-Westphalia/Germany in the course of time (Fürst, 2004).](http://www.efsa.eu.int)

**α-** and **γ-HCH** are nowadays normally no longer detectable at a detection limit of 0.001 mg/kg fat (Fürst, 2004). The ban of technical HCH in 1978 and thus the phasing out of the
The persistent isomers α- and β-HCH has clearly caused a decrease in human body burden and consequently in exposure of HCHs to breast fed babies. Assuming an average daily intake of 800 ml breast milk with a fat content of 3.5 %, the actual concentration of 0.020 mg β-HCH/kg fat would result in an average daily intake of 112 ng β-HCH/kg b.w. for a fully breast fed infant weighing 5 kg.

Analysis of 3500 human milk samples collected from women living in Northern Germany also showed a significant time-dependent decrease of median β-HCH levels. In 1995/1997 the median β-HCH level was 0.036 mg/kg fat, which was about 80 % lower than in 1986 (Schade and Heinzow, 1998). Comparable levels and declines in levels of HCHs in human milk by time have also been found in other European countries, such as Sweden (Atuma et al., 1998, Norén and Meironyte, 2000) and Norway (Polder et al., 2004). A recent survey which included several hundred nursing women in the UK neither did detect β-HCH at a limit of detection of 0.10 mg/kg fat nor α- and γ-HCH at a limit of detection of 0.01 mg/kg fat (Suremilk, 2004). In contrast, human milk samples from areas where HCHs were manufactured or production and application of technical HCH was banned later than 1978 show significantly higher levels of HCHs. This is the case for the region of Bitterfeld (former German Democratic Republic) where technical HCH and γ-HCH were produced and used until 1982 (Doering et al., 1999) as well as for a number of East European and developing countries where the levels of HCHs in humans are sometimes one to two orders of magnitude higher compared with Central Europe.

In the framework of the 3rd WHO human milk field study (Malisch et al., 2004) α-, β- and γ-HCH were analysed in 16 human milk pools from 10 European countries (Bulgaria, Czech Republic, Germany, Ireland, Italy, Luxembourg, Norway, Russia, Spain and Ukraine) and 11 pools from 6 non-European countries (Brazil, Egypt, Fiji, Hong Kong, Philippines and USA). Except for the pools from Bulgaria, Russia and Ukraine which showed levels between 0.002 and 0.006 mg α-HCH/kg lipid, α-HCH could not be detected in the European samples at a limit of detection of 0.001 mg/kg lipid. In European countries the levels for β-HCH ranged from 0.011 to 0.279 mg/kg lipid and for γ-HCH between < 0.001 and 0.013 mg/kg lipid. The highest levels in the European samples were found in the pools from Ukraine (α-HCH: 0.006 mg/kg lipid, β-HCH: 0.279 mg/kg lipid, γ-HCH: 0.013 mg/kg lipid) and Russia (α-HCH: 0.002 mg/kg lipid, β-HCH: 0.153 mg/kg lipid, γ-HCH: 0.001 mg/kg lipid). Considerably higher levels were found in 2 pooled human milk samples from Hong Kong which contained β-HCH at 1.32 and 1.36 mg/kg lipid, respectively.
CONCLUSIONS

Composition and environmental fate

• Depending on the product used, technical grade HCH or \( \gamma \)-HCH, and the time since last application before harvest, different HCH isomers can be found in varying ratios and concentrations in feed and food samples. The isomer ratio in a given sample can give valuable information about the composition of the applied product.

• Because of the lipophilic properties and persistence in the environment, \( \beta \)-HCH followed by \( \alpha \)-HCH and to a lesser extent \( \gamma \)-HCH may give rise to bioaccumulation and biomagnification through the food chain.

Adverse effects in animals

• No oral toxicity studies on \( \gamma \)- or \( \beta \)-HCH have been conducted in fish. These species are relatively sensitive to \( \gamma \)-HCH exposure through water and less sensitive to \( \alpha \)- and \( \beta \)-HCH exposure. The dose-response curve for \( \gamma \)-HCH in fish is steep. One study of oral toxicity of \( \alpha \)-HCH in rainbow trout indicates low susceptibility to this isomer. No effect was found at the highest dose tested, 1250 mg/kg feed for 3 months, which is 60,000 times higher than the current maximum level for \( \alpha \)-HCH in feed.

• The few data available on effects of \( \gamma \)-HCH in ruminants indicate relatively high sensitivity of these species. In sheep dosed 1.25 mg \( \gamma \)-HCH/kg feed for 6 months immune suppression was observed. This dose level is only 6 times above the current maximum level for \( \gamma \)-HCH in feed.

• Two studies on \( \gamma \)-HCH in pigs did not show toxic effects. The highest doses tested, 500 mg/kg feed for 2 months, and 80 mg/kg feed for 9 months, were respectively 2500 and 400 times above the current maximum level for \( \gamma \)-HCH in feed.

• In a 27-days study in chickens a NOAEL for \( \gamma \)-HCH of 4 mg/kg feed, has been identified. This is 20 times the current maximum level for \( \gamma \)-HCH in feed. In laying hens the NOAEL in a 60-days study was 10 mg/kg feed, this dose was 50 times the current maximum level for \( \gamma \)-HCH in feed.

• No effects of \( \beta \)-HCH have been found in chicken up to a concentration of 625 mg/kg in feed for 3 months. This is 60,000 times above the current maximum level for \( \beta \)-HCH in feed.

• In a 2-year study in dogs a NOAEL for \( \gamma \)-HCH of 50 mg/kg feed was identified.

• There is a lack of dose - response data after oral exposure to \( \alpha \)-HCH in livestock and pets, and of \( \beta \)-HCH in all relevant species.

Contamination of feed and carry over
Data on occurrence in various feed categories including fish feed, indicate low levels (in the low μg/kg range) of α-, β- and γ-HCH. However, because of the global trade of feedingstuffs and feed ingredients, products from regions with ongoing or recent use of HCHs may be more contaminated than products from areas where technical HCH and lindane have been banned for several years.

Following oral administration HCHs are rapidly and extensively absorbed in all animals tested, with the highest concentrations found in fat. The metabolism is extensive and HCHs are metabolised and mainly excreted as conjugate metabolites in urine. Half-lives vary from two days to one week for γ-HCH, to two to three weeks for α-HCH and to several weeks depending on species for β-HCH.

Half-lives vary from two days to one week for γ-HCH, to two to three weeks for α-HCH and to several weeks depending on species for β-HCH.

In ruminants, transfer of γ-HCH to milk is low, i.e. 0.7 - 3 % of ingested dose. For α-HCH and β-HCH the carry over was 4.7 and 30 - 37 %, respectively.

Human exposure

Data from European Countries, which banned the production and use of technical HCH at an early stage, indicate a permanent decline of HCH exposure to humans. Market basket studies performed between 1994 and 2003 in the Czech Republic indicate a significant decline of approximately 60 % for the average daily intake of HCH isomers. Human milk monitoring programmes in various countries revealed a corresponding decline of β-HCH levels up to 80 % since the 1980s. In current human milk samples α- and γ-HCH are only found occasionally.

Considering the decreasing concentration of HCHs in breast milk in some European countries, current human exposure through food in the European Union is likely to be very low, in the lower range of 1 – 10 ng/kg b.w./day. In contrast, human milk samples from some East European and developing countries with a more recent use of technical HCH show higher levels, indicating a higher exposure.

RECOMMENDATIONS

A European reporting system, allowing for exposure assessment of undesirable substances in feed is missing, and detailed data describing the background contamination of feedingstuffs and food of animal and plant origin are lacking.

Concentration levels for individual substances rather than condensed summaries for compound groups would be mandatory for a better understanding of the occurrence situation of undesirable substances in different feed materials and compound feeds as a prerequisite for a meaningful risk assessment and finally for a derivation of a possible temporal trend of the respective compounds in the feed chain.
REFERENCES


**SCIENTIFIC PANEL MEMBERS**


**ACKNOWLEDGEMENT**

The Scientific Panel on Contaminants in the Food Chain wishes to thank Jan Alexander, Aksel Bernhoft, George Bories, Jean-Pierre Cravedi, Peter Fürst, Niklas Johansson and Hans Schenkel for the contributions to the draft opinion.

**DOCUMENTATION PROVIDED TO EFSA**

**Submission of occurrence data**

Belgium

Czech Republic

Denmark

European Feed Manufacturers' Federation.

Germany

The Netherlands