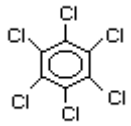
HEXACHLOROBENZENE (HCB)

Hexachlorobenzene (HCB) (CAS No.: 118-74-1) is a chlorinated aromatic hydrocarbon with the chemical formula C_6Cl_6

Chemical structure



Chemical structure of hexachlorobenzene (WHO-IPCS, 1997)

Properties

Hexachlorobenzene (HCB) is a chlorinated hydrocarbon which may contain some higher polychlorinated dibenzofurans and dioxins as impurities. 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 (EFSA, 2006).

Table 1: Properties of hexachlorobenzene (EFSA, 2006)

Solubility	5 - 6 µg/L. HCB is virtually insoluble in water at 25°C but slightly to very soluble in most organic solvents
Melting point	230°C
Henry's law constant	58.8 and 131 Pa m3/mol
log Koc	3.6 – 6.1
log Kow	5.2 – 6.5
Vapour pressure	2.3 x 10-3 Pa at 25°C

Contamination source

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 for seed treatment, especially for control of bunt of wheat. 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. 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 (EFSA, 2006).

HCB had other applications. It was involved in several industrial processes: as fluxing agent in aluminum smelting, as a regulator of porosity in the manufacture of graphite electrodes, as peptizing agent of rubber. It was used for manufacture of military pyrotechnics and went into the composition of wood preservatives. HCB was a synthetic intermediate in the production of certain rubber and chlorinated aromatic compounds (INERIS, 2005). 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 (EFSA, 2006).

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) and the United Nations Economic Commission for Europe (UNECE) Convention on long-range transboundary air pollution protocol on POPs (CLRTAP-POP).

HCB is ubiquitous in the environment, and has been measured in environmental and biological samples world-wide (EFSA, 2006).

The production and use of hexachlorobenzene have decreased since the 1970s owing to bans and restrictions on its use in many countries, but it still occurs as a by-product of the production of a number of chlorinated solvents and other industrial chemicals. Occupational exposure to hexachlorobenzene has occurred during its production and use in industry and agriculture.

Hexachlorobenzene has been detected in many foodstuffs, but dietary intake has probably decreased in recent years (IARC, 2001).

Incineration is an important source today of HCB in the environment (EFSA, 2006).

Analytical method

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 (EFSA, 2006). 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 (EFSA, 2006).

Toxicity

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 (EFSA, 2006).

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 (EFSA, 2006).

Metabolism

Hexachlorobenzene is lipophilic, accumulates in humans and is excreted as a cysteine conjugate of pentachlorobenzene. In rats, hexachlorobenzene has been shown to follow several metabolic pathways, which include the formation of pentachlorobenzene, tetrachlorobenzene and tri- and tetrachlorophenol (IARC, 2001).

Acute toxicity

HCB has low acute toxicity in experimental animals with LD50 values being in the range of 1000 to 10,000 mg/kg bw per day for various species. Acute lethal doses trigger convulsions, tremor, ataxia and paralysis (EFSA, 2006). Accidental consumption by humans of a large quantity of hexachlorobenzene resulted in porphyria cutanea tarda, liver toxicity, neurological effects and skin changes, which were persistent (IARC, 2001).

In humans, manifestations of disturbances in porphyrin metabolism were observed in accidental poisonings that took place in Turkey from 1955 to 1959 (EFSA, 2006).

Repeated toxicity

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).

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.

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

body weight (bw)/day, rats showed mild effects in the liver (proliferation of smooth endoplasmatic reticulum, altered mitochondria and increased number of storage vesicles, enzyme induction); the NOAELs in these studies were 0.05 to 0.07 mg HCB/kg bw/day. Changes in bone structure associated with disturbed calcium homoeostasis were observed in sub-chronic studies in rats at 0.7 mg HCB/kg bw/day, but not at 0.07 mg/kg bw/day. The lowest NOAEL, 0.05 - 0.07 mg/kg bw for hepatic effects were found in a subchronic study in pigs and several chronic studies in rats WHO-IPCS 1997). Beagle dogs were much less sensitive to hepatotoxicity.

Carcinogenicity
The risk for breast cancer has been investigated in relation to life-long, accumulated exposure to hexachlorobenzene in nine studies.

Hexachlorobenzene was tested for carcinogenicity by oral administration in one study in mice, four studies in rats and one in hamsters. It produced liver-cell tumours in all three species and renal tubular tumours in rats of each sex in one study. After perinatal administration to rats, it increased the incidences of parathyroid adenomas in males and adrenal phaeochromocytomas in females. In hamsters, it also produced liver hepatomas, haemangioendotheliomas and thyroid follicular-cell adenomas. In several studies in which it was given with other compounds, hexachlorobenzene promoted liver carcinogenesis 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 and EFSA, 2006).

Hexachlorobenzene has been evaluated by the International Agency for Research on Cancer (IARC, 2001). It was concluded that there is *inadequate evidence* in humans for the carcinogenicity of hexachlorobenzene; There is *sufficient evidence* in experimental animals for the carcinogenicity of hexachlorobenzene. Hexachlorobenzene is *possibly carcinogenic to humans (Group 2B)*.
In the European Union, HCB was classified as Carc. Cat.2; R45 according to Dir 67/548/EEC and as Carc.1B H350 according to CLP Regulation (EC) No. 1272/2008.

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).
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 (EFSA, 2006).

In a single study of workers exposed to a number of chlorinated solvents, including hexachlorobenzene, an increased frequency of micronucleated lymphocytes was found; there was no association with the concentrations of hexachlorobenzene in blood. Micronuclei were induced by hexachlorobenzene in human and rat primary hepatocytes *in vitro*. Otherwise, there was little evidence that hexachlorobenzene has genetic activity (IARC, 2001).

In experimental animals, the effects of treatment with hexachlorobenzene on the thyroid include decreased thyroid hormone concentrations due to increased glucuronidation and inhibition of type-1 deiodinase, interference with serum carrier binding of the thyroid hormones and increased thyroid-stimulating hormone concentrations. In the livers of experimental animals, hexachlorobenzene induced cytochrome P450 enzymes and inhibited uroporphyrinogen decarboxylase, iron accumulation and oxidative damage. These effects are believed to be involved in the production of hepatic tumours (IARC, 2001).

Reproductive toxicity
In a poisoning epidemic in Turkey, exposure to hexachlorobenzene via breast milk caused a very high rate of lethality among infants. An increased frequency of pregnancy loss was reported among

women exposed to hexachlorobenzene as children. The presence of this compound in breast milk has been associated with altered immune function in Inuits. Hexachlorobenzene was teratogenic in mice, and increased mortality rates were observed among rats and monkeys exposed *in utero*. Effects on steroid hormones have also been reported in exposed female mice (IARC, 2001, EFSA 2006).
Male reproductive functions as studied in mice, rats and pigs were affected at higher doses (e.g. 30 mg/kg bw/day for 21 days in mice, 50 mg/kg bw/day for 90 days in pigs and 221 mg/kg bw/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 bw/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 bw/day 90 days prior to mating and throughout gestation and lactation (IARC, 2001; WHO-IPCS, 1997).

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 bw/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.

Establishment of Health Based Reference Values
In 1997, IPCS suggested a health based guidance value of 0.17 µg/kg bw per day for non neoplastic effects based on a NOAEL for 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).
The approach for neoplastic effect is based on the tumorigenic Dose 5, or TD_5 i.e., the intake or exposure associated with a 5% excess incidence of tumours in experimental studies in animals. The TD_5 values range from 0.81 mg/kg bw/day for neoplastic liver nodules in females of the 2-generation carcinogenicity study in rats to 2.01 mg/kg bw/day for parathyroid adenomas in males (WHO-IPCS, 1997).

EFSA (2006) 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 $BMDL_{10}$ (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.

EPA established a TDI of 0.0008 mg/kg bw/day (Nougadère et al., 2011).

Occurrence in food

HCB is primarily found in fatty food. Fish and fish oil are known to contain high level of HCB.
Concentration in organochlorine pesticides measured is fish in Belgium in 2005-2006 are generally under the reporting limit (Vromman et al., 2008).

Mean cconcentration of HCB measured for commercially Atlantic salmon fillets on the Norwegian market in 2007 is 1.4 ng/g ww (range 0.8–3.5, n =27) (Berntssen et al., 2011).
Mean concentration of HCB (sum) measured in ponds in Lorraine Region (France) in 2008 is 0.42 (0.10–0.80) ng/g wet weight in carp (n=25) (Thomas et al., 2012).
Concentration of HCB measured in eels in Scotland (2004-2008) range from 3 to 7.2 µg/kg wet weight (Macgregor et al., 2010). Concentration of HCB measured by Szlinder-Richert et al. (2010) in eels in Poland ranged between 0.4 and 23.8 ng/g ww.

Content of HCB in food samples taken by Fromberg et al. (2011) from 1998 to 2003 in Denmark are presented in table 2.

Table 2: Content of HCB in food samples taken by Fromberg et al. (2011) from 1998 to 2003 in Denmark

Foodstuff	**Mean HCB (µg/kg fish and egg and µg/kg fat for other foods).**
Chicken fat	0.7
Turkey fat	0.8
Other poultry fat	5.6
Beef fat	3.0
Pork fat	0.6
Lamb sheet fat	5.2
Milk, Danish	2.2
Milk, foreign	1.7
Cheese, Danish	2.3
Cheese, foreign	2.3
Butter, Danish	0.9
Butter, foreign	1.0
Butter fat, mixed	0.4
Eggs	1.0
Eel, farmed, raw	5.2
Greenland halibut, raw	3.6
Herring, raw	0.8
Herring, pickled	1.2
Herring, smoked	0.9
Lumpsucker, raw	4.1
Mackerel, raw	0.9
Mackerel, smoked	1.0
Mackerel, tinned in tomato	0.4
Rainbow trout, farmed, raw	0.8
Salmon, raw	1.6
Swordfish, raw	0.4
Trout, marine farmed, raw	1.3
Fish oil	3.9
Cod liver oil	10.5

HCB was detected in 86% of the Belgian mothers human milk collected in 2006 during the fourth World Health Organization Human biomonitoring campaigns (Colles et al., 2008). Mean concentration was 15.2 +/- 7.6 ng/g fat.

Dietary exposure assessment

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 (EFSA, 2006).

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 bw/day which is two to three orders of magnitude below the suggested health based guidance value of 170 ng/kg bw per day (EFSA, 2006).

Fromberg et al. (2011) have estimated the dietary intake of HCB for Danish adults to 1.3 ng/kg bw/day (mean), 1.9 ng/kg bw/day (P90) and 2.3 ng/kg bw/day. Calculated estimation for children was 2.6 ng/kg bw/day (mean), 4.0 ng/kg bw/day (P90) and 4.8 ng/kg bw/day (P95).

Mean daily intake for adult reported by Guatemala, Japan, The Netherlands, the United Kingdom and the United States were below 0.025 µg/kg bw (Ahmed, 1999).

Risk characterization

The margin between the dose causing a 5% increase above background of liver tumours in rats (0.81 mg/kg bw) and the human exposure range as given above is 1.6 – 80 x 10^5, which would indicate low concern from a public health point of view (EFSA, 2006).
Table 3 present the HCB dietary exposure of the Danish population from Fromberg et al. (2011) and the calculated MOE. The values are in the range given by EFSA (2006).

Table 3: HCB dietary exposure for adult and children and calculated MOE (dietary exposure data come from Fromberg et al., 2011)

Population	Dietary exposure (ng/kg bw/day)	MOE (TD_5: 0.81 mg/kg bw)
Danish adults - mean	1.3	6.23E+05
Danish adults - P90	1.9	4.26E+05
Danish adults - P95	2.3	3.52E+05
Danish children - mean	2.6	3.12E+05
Danish children - P90	4	2.03E+05
Danish children - P95	4.8	1.69E+05

Nougadère et al. (2011) have calculated an EDI (mean % of TDI) between 0.0 and 13.9% for the French children and between 0.0 and 12.9% for the French adult based on a TDI of 0.0008 mg/kg bw/day.

Legislation

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 which will repeal the four Council Directives

Recommendations

The CONTAM Panel of EFSA made the following recommendations for HCB in feed (EFSA, 2006):

- 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 noncompliant 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.

References

Ahmed F. E. 1999. Safety standards for food contaminants pp. 545. in Environmental contaminants in food. Edited by Colin F. Moffat and Kevin J. Whittle. USA.

Berntssen M. H.G., Maage A., Julshamn K., Oeye B.E., Lundebye A.-K. 2011. Carry-over of dietary organochlorine pesticides, PCDD/Fs, PCBs, and brominated flame retardants to Atlantic salmon (Salmo salar L.) fillets. Chemosphere 83, 95–103.

Colles A., Koppen G., Hanot V., Nelen V., Dewolf M.-C., Noël E., Malisch R., Kotz A., Kypke K., Biot P., Vinkx C., Schoeters G. 2008. Fourth WHO-coordinated survey of human milk for persistent

organic pollutants (POPs): Belgian results. Chemosphere, 73, 907–914.
EFSA. 2006. Opinion of the Scientific panel on contaminants in the food chain on request from the Commission related to hexachlorobenzene as undesirable substance in animal feed. The EFSA Journal 402, 1 – 49.
IARC (International Agency for Research on Cancer). 2001. Hexachlorobenzene. Summaries and Evaluations 79: 493. http://www.inchem.org/documents/iarc/vol79/79-13.html
Fromberg A., Granby K., Højgård A., Fagt S., Larsen J.C. 2011. Estimation of dietary intake of PCB and organochlorine pesticides for children and adults. Food Chemistry, 125, 1179–1187.
INERIS. 2005. Hexachlorobènzene. Données technico-économiques sur les substances chimiques en France http://www.ineris.fr/rsde/fiches/fiche_hexachlorobenzene.pdf
Macgregor K., Oliver I. W., Harris L., Ridgway I. M. 2010. Persistent organic pollutants (PCB, DDT, HCH, HCB & BDE) in eels (*Anguilla anguilla*) in Scotland :Current levels and temporal trends. Environmental Pollution, 158, 2402-2411.
Nougadère A., Reninger J.-C., Volatier J.-L., Leblanc J.-C. 2011. Chronic dietary risk characterization for pesticide residues: A ranking and scoring method integrating agricultural uses and food contamination data. Food and Chemical Toxicology, 49, 1484–1510.
Szlinder-Richert J. Usydus Z., Pelczarski W. 2010. Organochlorine pollutants in European eel (Anguilla anguilla L.) from Poland. Chemosphere, 80, 93–99.
Thomas M., Lazartigues A. l., Banas D., Brun-Bellut J., Feidt C. 2012. Organochlorine pesticides and polychlorinated biphenyls in sediments and fish from freshwater cultured fish ponds in different agricultural contexts in north-eastern France. Ecotoxicology and Environmental Safety 77, 35–44.
Vromman V., Rettigner C., Huyghebaert A., Maghuin-Rogister G., Bossier P.,Delbare D., Parmentier K., Van Camp J., Verbeke W., Vinkx C., Pussemier L. L'aquaculture : production, alimentation et présence de contaminants environnementaux et de résidus de médicaments vétérinaires. Ann. Méd. Vét., 2008, 152, 227-239.
WHO-IPCS (World Health Organization – International Programme on Chemical Safety). 1997. Hexachlorobenzene, Environmental Health Criteria 195. World Health Orgaization, Geneva, Switzerland. http://www.inchem.org/documents/ehc/ehc/ehc195.htm.