Hexachlorobenzene is also a dioxin-like compound: Possible impact on the TEQ

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What properties do dioxin-like compounds have?

A dioxin-like compound is a compound that binds to the Aryl hydrocarbon (Ah) receptor, results in dioxin-like effects, and bioaccumulates[1].

Does hexachlorobenzene have these properties?

- Binding to the Ah-receptor: HCB has an affinity for the Ah-receptor 10,000 times less than TCDD[2]. This is in the same range as the mono-ortho substituted PCBs 2,3,3',4,4'-pentachlorobiphenyl (PCB 105), 2,3',4,4',5-pentachlorobiphenyl (PCB 118), and 2,3,3',4,4',5-hexachlorobiphenyl (PCB 156)[3].
- Dioxin-like effects: Exposure to HCB results in dioxin-like effects, such as induction of hepatic cytochrome P4501A1 (CYP1A1) and P4501A2 (CYP1A2) activities, hepatic porphyrin accumulation and excretion, alterations in thyroid hormone levels and metabolism, alterations in retinoid levels, liver damage, reduction in reproduction, splenomegaly, increase in mortality, neurological alterations, teratologic effects, and immunotoxic effects[4]. Some of these effects can not be classified as unique for TCDD-like compounds, such as porphyrin accumulation and a decrease in circulating thyroid hormone levels since it has been shown that multiple mechanisms are involved in these responses[5,6]. HCB is a mixed type inducer having also the properties of phenobarbital-like effects, such as induction of hepatic cytochrome P4502B (CYP2B) activity[7,8]. Recently, it has been shown that CYP1A2 (-/-) knockout mice did not develop uroporphyria after exposure to HCB, whereas cyp1A2 (+/+) wildtype mice developed uroporphyria[9]. This clearly indicates that the involvement of CYP1A2 is needed for a porphyrinogenic response.
- Bioaccumulation: The bioaccumulation of HCB can be found in the long half-life in various species (ranging from weeks to years), the high log octanol/water partition coefficient (5.5), and the biomagnification of HCB in various studies in natural aquatic ecosystems[4]. For example, the (whole body) half-life of HCB in male Wistar rats has been reported to be 20 days[10]. In male Sprague Dawley rats and male white rabbits, the half-life was calculated to be 24 days and 32 days, respectively[11]. In rhesus monkeys, the half-life for HCB has been estimated to be 2.5 to 3 years[12]. No data on the half-life on HCB in humans are available.

In other words, hexachlorobenzene has all three properties.

Can a relative potency value for hexachlorobenzene be estimated?

The WHO uses a tiered approach for estimating TEF values, giving long-term *in vivo* studies the preference over short-term *in vivo* studies, which have a priority over *in vitro* studies or structure-activity considerations[1]. Unfortunately, no *in vivo* studies designed for estimating a TEF value are available. The *in vitro* data available were derived from chicken hepatocytes with HCB and TCDD[13]. The EC50s for HCB and TCDD in this system were determined for ethoxyresorufin

O-deethylase activity and accumulation of uroporphyrin and were expressed as the concentration at which 50% of the maximum effect was reached. The uroporphyrin accumulation in this specific system was not dependent on the rate-limiting enzyme δ-aminolevulinic acid synthetase, suggesting that only an Ah-receptor mediated mechanism was involved. Based on the EC50s of these two endpoints, the relative potency for HCB was estimated to range from 0.00006 to 0.0002 (Table 1). Another study in which HCB was compared to TCDD includes binding to the Ah-receptor, which was reported to be 10,000 times less than TCDD[2]. Taken all these data together it can be estimated that HCB has a relative potency value of 0.0001 (Table 1).

Possible impact of inclusion of hexachlorobenzene in Toxic Equivalency Factor concept

The daily intake by infants via breast milk is calculated to range from less than 0.018 to 5.1 µg/kg body weight in various countries[4]. Using a relative potency value of 0.0001 for HCB, this equals to less than 1.8 to 510 pg TEQ/kg/day. The concentration of HCB in breast milk samples covers a wide range with having the highest concentration in breast milk samples from Spain, the Czech Republic, Slovakia, and India[14]. Lipid adjusted HCB levels range from 0.007 to 5 mg/kg human milk. Using a relative potency value of 0.0001, these HCB values range from 0.7 to 500 ng TEQ/kg lipid. For comparison, the concentration of PCDDs, PCDFs, and PCBs together range from about 10 to 45 ng TEQ/kg lipid[14]. This indicates that in most countries with lower HCB levels in human milk the contribution of HCB to the total TEQ can add an additional 10 to 60% to the total TEQ (Canada, Denmark, Faroe Islands, Germany, Japan, Kazachstan, The Netherlands, and the U.S.). However, of most concern are the high levels of HCB in human milk in the Czech Republic, Slovakia, and Spain, adding up to six times the dioxin-activity in human milk in comparison to the contribution of PCDDs, PCDFs, and PCBs together expressed as TEQ. For a breast-fed infant consuming 150 mL milk per kilogram body weight per day, this could be as high as 1 ng TEQ/kg/day. The HCB levels in human milk in these countries are about the same as in India.

Conclusion

It has clearly been shown that based on binding to the Ah-receptor, the dioxin-like effects, and the bioaccumulation in higher trophic levels, HCB should be classified as a dioxin-like compound. The mechanism of action resembles that of mono-ortho substituted PCBs, which have also phenobarbital-like properties and are included in the TEF concept. Based on the limited information available it was estimated that HCB is about 10,000 times less potent than TCDD. Using a relative potency value of 0.0001, HCB could add 10-60% to the total TEQ in human milk samples in most countries. In a few countries such as Spain, Slovakia, and the Czech Republic, HCB levels in human milk expressed as TEQ could be six-fold than the total TEQ based on PCDDs, PCDFs, and PCBs together. The HCB levels in human milk in these countries are about the same as in India. These studies clearly indicate that more studies are needed to reduce the uncertainty in the estimation of a relative potency value for HCB and that epidemiological studies should be undertaken in infants fed breast milk in countries with high HCB exposure levels. Furthermore, measurements of HCB levels in human and environmental samples in conjunction with other dioxin-like compounds are a prerequisite to estimate the total dioxin-activity in these samples.

Table 1. Relative potency estimates for hexachlorobenzene (HCB).

Effect	TCDD	HCB	Relative potency	Reference

			for HCB	
Binding affinity to the Ah receptor (nM)	0.18	2100	0.00009	(*2*)
EC50 for EROD induction in chicken hepatocytes (nM)	0.014-0.020	130-150	0.00009-0.0002	(*13*)
EC50 for accumulation of uroporphyrin in chicken hepatocytes (nM)	0.002-0.004	25-35	0.00006-0.0002	(*13*)

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