Literature Search Product for Hexabromocyclododecane

Results of the June 2007 literature search arranged within the framework of an IRIS Toxicological Review (Chapter 2 through Section 4.5)

TOXICOLOGICAL REVIEW OF HEXABROMOCYCLODODECANE (CAS No. 3194-55-6, 25637-99-5, and 25495-98-1)

The citations marked ● are review articles that will be examined for relevant material. Information contained therein may provide input to several of the chapters and sections listed below.

● BASF. (1996) Prüfempehlung BUA-Stoffbericht "Hexabromcyclododecan" (HBCD). Complementary report 165.

● Birnbaum, LS; Staskal, DF. (2004) Brominated flame retardants: cause for concern? Environ Health Perspect 112:9–17.
Brominated flame retardants (BFRs) have routinely been added to consumer products for several decades in a successful effort to reduce fire-related injury and property damage. Recently, concern for this emerging class of chemicals has risen because of the occurrence of several classes of BFRs in the environment and in human biota. The widespread production and use of BFRs; strong evidence of increasing contamination of the environment, wildlife, and people; and limited knowledge of potential effects heighten the importance of identifying emerging issues associated with the use of BFRs. In this article, we briefly review scientific issues associated with the use of tetrabromobisphenol A, hexabromocyclododecane, and three commercial mixtures of polybrominated diphenyl ethers and discuss data gaps. Overall, the toxicology database is very limited; the current literature is incomplete and often conflicting. Available data, however, raise concern over the use of certain classes of brominated flame retardants

● Covaci, A; Gerecke, AC; Law, RJ; et al. (2006) Hexabromocyclododecanes (HBCDs) in the environment and humans: A review. Environ Sci Technol 40:3679–3688.
Hexabromocyclododecanes (HBCDs) are brominated aliphatic cyclic hydrocarbons used as flame retardants in thermal insulation building materials, upholstery textiles, and electronics. As a result of their widespread use and their physical and chemical properties, HBCDs are now ubiquitous contaminants in the environment and humans. This review summarizes HBCD concentrations in several environmental compartments and analyzes these data in terms of point sources versus diffuse sources, biomagnification potential, stereoisomer profiles, time trends, and global distribution. Generally, higher concentrations were measured in samples (air, sediment, and fish) collected near point sources (plants producing or processing HBCDs), while lower concentrations were recorded in samples from locations with no obvious sources of HBCDs. High concentrations were measured in top predators, such as marine mammals and birds of prey (up to 9600 and 19 200 ng/g lipid weight, respectively), suggesting a biomagnification potential for HBCDs. Relatively low HBCD concentrations were reported in the few human studies conducted to date (median values varied between 0.35 and 1.1 ng/g lipid weight). HBCD levels in biota are increasing slowly and seem to reflect the local market demand. One important observation is the shiftfrom the high percentage of the gamma-HBCD stereoisomer in the technical products to a dominance of the alpha-HBCD stereoisomer in biological samples. A combination of factors such as variations in solubility, partitioning behavior, uptake, and, possibly, selective metabolism of individual isomers may explain the observed changes in stereoisomer patterns. Recommendations for further work include research on how HBCDs are transferred from products into the environment upon production, use, and disposal. Time trends need to be analyzed more in detail, including HBCD stereoisomers, and more data on terrestrial organisms are needed, especially for humans. Whenever possible, HBCDs should be analyzed as individual stereoisomers in order to address their fate and effects

● Darnerud, PO. (2003) Toxic effects of brominated flame retardants in man and in wildlife. Environ Int 29:841–853.
Brominated flame retardants (BFRs) are ubiquitous industrial chemicals, and many of them are produced in large

volumes. Due to this fact, several BFRs are found in quantifiable levels in wildlife, as well as in humans. However, we are still lacking information on the effects of BFR in wildlife and, especially, in man. This review summarizes the biological effects of polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA) and derivates, hexabromocyclododecane (HBCD) and polybrominated biphenyls (PBBs), however excluding other aspects such as environmental levels. These BFR groups were selected because of a large volume production (PBDEs, TBBPA and derivates), and availability of some toxicity data in spite of much lower production volumes (HBCD and PBBs). In addition, the increase in levels of PBDEs in human (breast milk) and wildlife samples during later time made it especially interesting to include this BFR group. PBDES: The commercial PBDE products predominantly consist of so-called penta-, octa- and decabromodiphenyl ether products. Each product consists of a rather narrow range of congeners and is named after the dominating congener as regards the bromination pattern. Generally, the PentaBDEs seem to cause adverse effects at the comparably lowest dose, whereas much higher doses were needed for effects of the DecaBDEs. The critical effects of PentaBDEs are those on neurobehavioural development (from 0.6 mg/kg body weight) and, at somewhat higher dose, thyroid hormone levels in rats and mice, of OctaBDEs on fetal toxicity/teratogenicity in rats and rabbits (from 2 mg/kg body weight), and of DecaBDEs on thyroid, liver and kidney morphology in adult animals (from 80 mg/kg body weight). Carcinogenicity studies, only performed for DecaBDEs, show some effects at very high levels, and IARC (1990) evaluates DecaBDEs not classifiable as to its carcinogenicity to humans. TBBPA: The toxicity of TBBPA in the experimental in vivo studies is suggested to be low. In most reported studies, only doses in g/kg body weight were effective, but at least one study suggested renal effects at around 250 mg/kg body weight. Although difficult to include and interpret in a quantitative risk assessment, the in vitro effects on immunological and thyroid hormones, as well as binding to erythrocytes should be noted. Before a solid standpoint could be reached on TBBPA toxicity additional studies must be performed. This statement is even more valid regarding the TBBPA derivates, where there is an almost complete lack of toxicity data. HBCD: Also in the case of HBCD, relevant toxicity studies are lacking. Based on the present animal studies, a critical effect is seen in the liver and on thyroid hormones (LOAEL 100 mg/kg body weight/day). However, in a recent short paper behavioural effects in mice pups were observed already at 0.9 mg/kg body weight, and behavioural effects may be a sensitive endpoint for HBCD, as well as for other BFRs. PBBS: Due to the Michigan accident in 1973-1974, many toxicity studies on PBBs are available. The critical experimental effects are those on reproduction and carcinogenicity, and a NOAEL of 0.15 mg/kg body weight/day could be suggested based on the cancer effects. In man no unequivocal effects have been observed, although in some studies neurological and musculoskeletal symptoms were suggested. Based on the carcinogenic effects in animals, a human TDI of 0.15 microg/kg body weight has been presented. To conclude, the toxicity data are almost entirely based on experimental models. There are differences among the BFR groups, as well as within these groups, both regarding type of toxic effect and at what dose it appears. As BFRs will continue to appear both in industrial applications and, even if the production has ceased, in our environment, there is a continued need for effects studies on BFRsde Wit, CA. (2000rdsverket;) Brominated flame retardants. Swedish Environmental Protection Agency (Naturv&Aring :94p,P. Brominated flame retardants are used in electronic appliances and textiles. They are detected in the environment and in breast milk in increasing concentrations. Their persistence is a matter of concern and poses a potential problem similar to that of polychlorinated biphenyls (PCBs). This report discusses these issues and future trends. Contents: characteristics of flame retardants; brominated flame retardant chemistry; analytical methods for brominated flame retardants; toxicology; environmental concentrations; future trends

● de Wit, CA. (2002) An overview of brominated flame retardants in the environment. Chemosphere 46:583–624. The presence of brominated flame retardant (BFR) chemicals, and particularly polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCD), has become of increasing concern to scientists over the past decade. Environmental studies conducted primarily in Europe, Japan and North America indicate that these chemicals are ubiquitous in sediment and biota. The levels of PBDEs seem to be increasing, and several trends, including in humans, indicate that this increase may be rapid. The occurrence of high concentrations of certain PBDE isomers may be sufficient to elicit adverse effects in some wildlife. There is also concern that levels could cause adverse effects in sensitive human populations such as young children, indigenous peoples, and fish consumers. However, our knowledge about these chemicals, their sources, environmental behavior, and toxicity is limited, making risk assessment difficult. In this paper, the current state of knowledge is reviewed and areas for further research recommended to improve future monitoring and risk assessment efforts

● Drohmann, D. (2006) HBCD: facts and insinuations. Environ Sci Technol 40:1

● European Commission. (2000) IUCLID Dataset CAS No. 3194-55-6. European Chemicals Bureau, European

Commission. Available online at http://ecb.jrc.it/esis/index.php?GENRE=CASNO&ENTREE=3194-55-6.

● European Commission. (2005) IUCLID Dataset CAS No. 25637-99-4. European Chemicals Bureau, European Commission. Available online at http://www.ecy.wa.gov/programs/eap/pbt/rule/docs/novcomments/BSEFhexa.pdf.

● Hexabromcyclododecan (Aug 1995) (1996) Beratergremium Fuer Umweltrelevante Altstoffe (BUA) 165

● Hardy, ML; Biesemeier, J; Manor, O; et al. (2003) Industry-sponsored research on the potential health and environmental effects of selected brominated flame retardants. Environ Int 29(6):793–799.
Modern fire-fighting techniques, equipment and fire-resistant building design has lead to less destruction than in the previous centuries. However, a high fuel load in either a residence or a commercial building can overwhelm even the best firefighters or building construction, and factors affecting the fuel load have changed in recent decades. The fire load in a typical home has doubled over the last 50 years, furnishings typically include those made of petrochemicals that can behave as if containing built-in accelerant, and modern energy-efficient buildings are less able to disperse heat in the event of a fire. Flame retardant chemicals (FRs) are one means used to reduce the risk of fire. FRs are typically added or incorporated chemically into a polymer to slow or hinder the ignition or growth of a fire in low-to-moderate cost commodity polymers. One type of FR contains bromine atoms as the active moiety. The FR industry, either as individual companies or as consortia, has conducted a broad range of studies on the commercial deca-, octa- and pentabromodiphenyl oxide/ether, tetrabromobisphenol A and hexabromocyclododecane products. These five products have data in excess of the OECD Screening Informational Data Set (SIDS) and the U.S. High Production Volume (HPV) program, and sufficient data for the performance of formal EU risk assessments. The objective of this paper is to present the range of data developed by industry consortia and to provide sources for the information. We hope to facilitate further research by assembling references to industry consortia-sponsored research here

● Ishizu S, Kawai T and Aoyagi M. (not published, translated into English). (undated report cited in SCI, 2007).

● KEMI (Kemikalieinspektionen). (1995) The flame retardants proj. Box 1384, 171 27 Solna, Sweden, 83p,P.
This document contains a collection of reports on the toxicity and ecotoxicity of selected brominated flame retardants and an updated ecotoxicological summary for tetrabromobisphenol A. The properties and toxic effects of the following flame retardants are reviewed: decabromodiphenyl oxide; pentabromoethylbenzene, pentabromomethylbenzene, tetrabromobisphenol A, ammonium bromide, N,N'-ethylenebis(tetrabromophthalimide), hexabromocyclododecane, tetrabromophthalic anhydride. There are no data available on the effects of these flame retardants on humans

● Kroschwitz JI (Editor). (1992) Kirk-Othmer Enclycopedia of chemical technology Fourth Edition. 10, pp 964 John Wiley & Sons.

● Kruse, H; Paulsen, O; Schau, C; et al. (2000) A German toxicology review document--Hexabromocyclododecane (HBCD) starts on p. 92. Germany. Available online at http://www.umweltdaten.de/publikationen/fpdf-l/1967.pdf.

● Law, RJ; Kohler, M; Heeb, NV; et al. (2005) Hexabromocyclododecane challenges scientists and regulators. Environ Sci Technol 39(13):281A–287A

● Liepins, R; Pearce, EM. (1977) Chemistry and toxicity of flame retardants for plastics. Environ Health Perspect 17:55-63.
An overview of commercially used flame retardants is given. The most frequently used flame retardants are illustrated and the seven major markets, which use 96% of all flame-retarded polymers, are described. The annual flame retardant growth rate for each major market is also projected. Toxicity data are reviewed on only those compositions that are considered commercially significant today; this includes 18 compounds or families of compounds and 4 inherently flame-retarded polymers. Toxicologic studies of flame retardants for most synthetic materials are of recent origin and only a few of the compounds have been evaluated in any great detail. Considerable toxicologic problems may exist in the manufacturing of some flame retardants, their by-products, and possible decomposition products

● NRC (National Research Council). (2000) Toxicological risk of selected flame-retardant chemicals. In:

Toxicological Risks of Selected Flame-retardant Chemicals. National Academy Press; Washington, DC. pp. 53-71.

● Salomon, M. (2005) Brominated flame retardants - Status quo in risk discussion <Original> Bromierte flammschutzmittel - aktueller stand der risikodiskussion. Umweltmedizin in Forschung Und Praxis (UMWELTMED FORSCH PRAX) 10:183-197.
Brominated flame retardants (BFR) are a group of chemicals which are produced in large quantities and occur in many consumer products. The BFR which are currently quantitatively most dominant in Europe are tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCD) and the polybrominated diphenyl ether (PBDE). BFR are predominantly used as additives. They are released into the environment by using products and through depositing waste, as well as other means. In the meantime they are ubiquitously detectable. Their lipophilie and persistence encourage bioaccumulation. Besides occupational exposure, humans assimilate these substances primarily via ingestion. At present the internal exposure and the concentrations in foodstuff are very low. It is known that acute toxicity of the BFR is marginal, but they can disrupt the thyroid hormone system and the neurological development. The relevance of neurotoxic and endocrine effects for humans, determined in in vitro tests and animal experiments, is still uncertain. It is undisputed that pregnant women, developing foetuses and infants are especially sensitive to potential disruption of the thyroid hormone system. For this reason the presence of BFR in human breast milk should be noted. Overall the insights about the behaviour in the environment, in the organism and the effects in particular of TBBPA and HBCD are still rudimental. Due to the existent knowledge about potential risks from PBDE two of the three used commercial mixtures (penta-BDE and octa-BDE) are already regulated in Europe. The usage of deca-BDE is still in discussion. The bioaccumulative and toxic potential of this congener is very low, but there is strong evidence that it is transformed in the environment to more toxic compounds. (c) ecomed Medizin, Verlagsgruppe Huthig Jehle Rehm GmbH

SCI (Swedish Chemicals Inspectorate). (2007) Risk assessment report: hexabromocyclododecane [draft]. Produced by the Swedish Chemicals Inspectorate, Sundbyberg, Sweden, for the Institute for Health and Protection, European Chemicals Bureau, European Commission-Joint Research Centre, Luxembourg.

2. CHEMICAL AND PHYSICAL INFORMATION

Becher, G. (2005) The stereochemistry of 1,2,5,6,9,10-hexabromocyclododecane and its graphic representation. Chemosphere 58:989–991.
1,2,5,6,9,10-Hexabromocyclododecane, a widely used additive flame retardant, is produced by bromination of cis,trans,trans-cyclododeca-1,5,9-triene, resulting in a mixture of three enantiomeric pairs of diastereomers. We present here the correct configuration and graphic representation of the six isomers. Recently, the complete separation of all six isomers has been achieved using chiral liquid chromatography

Betts, K. (2005) More clues to HBCD isomer mystery. Environ Sci Technol 39:146A–147A

Cariou, R; Antignac, JP; Marchand, P; et al. (2005) New multiresidue analytical method dedicated to trace level measurement of brominated flame retardants in human biological matrices. J Chromatogr A 1100:144–152.
A new method has been developed for the multi-residue measurement of the main brominated flame retardants (alpha- and gamma-hexabromocyclododecane (HBCD), tetrabromobisphenol A (TBBP-A) and polybrominated diphenyl ethers including decabromodiphenyl ether) in human biological matrices (serum, adipose tissue and breast milk). The proposed sample preparation procedure focused on reduced solvent and consumable consumption and associated procedural contamination, as well as reduced sample size. This protocol was fully validated and was proved to be suitable for identification of brominated flame retardant residues at ultra-trace level, as attested by preliminary results on real samples

Hayward, SJ; Lei, YD; Wania, F. (2006) Comparative evaluation of three high-performance liquid chromatography-based Kow estimation methods for highly hydrophobic organic compounds: polybrominated diphenyl ethers and hexabromocyclododecane. Environ Toxicol Chem 25:2018–2027.
Three methods for estimating the octanol-water partition coefficient (Kow) based on its relationship with capacity factors on reversed-phase (RP) high-performance liquid chromatography (HPLC) columns were compared in terms of their applicability to highly hydrophobic compounds (HHCs). Methods based on simple isocratic elutions were found to be unsuitable, because the very high organic modifier fractions that are required to elute HHCs from RP

columns challenge the basic assumption of the similarity between the octanol-water and RP-eluent systems. Compound planarity was found to exert a considerable influence on the retention of HHCs in RP columns, leading to different linear calibration curves for chlorobenzenes and chlorobiphenyls. Only an empirical exponential regression succeeded in describing the behavior of both groups of compounds during gradient elutions. In a method based on isocratic retention times at multiple temperatures, satisfactory calibration was achieved with a multivariate linear regression that included a numerical indicator of compound planarity. Considering experimental simplicity, speed, precision, and accuracy, with the latter judged by comparison with Kow values for polybrominated diphenyl ethers and polychlorinated naphthalenes as reported in the literature, a gradient elution combined with an exponential calibration curve is recommended for estimating the Kow of HHCs. To our knowledge, the first isomer-specific Kow values for hexabromocyclododecane are reported. Bearing in mind that the influence of structural characteristics on retention is likely to increase with hydrophobicity, it is not justified to judge a HPLC-based Kow estimation method as being suitable for HHCs because it is shown to work well for less hydrophobic substances. Whereas univariate linear regressions often may prove to be sufficient when dealing with substances having a log Kow, of less than five, methods for HHCs need to account for the influence of structure on retention

Heeb, NV; Schweizer, WB; Kohler, M; et al. (2005) Structure elucidation of hexabromocyclododecanes--a class of compounds with a complex stereochemistry. Chemosphere 61:65–73.
Hexabromocyclododecanes (HBCDs) are high production volume chemicals (16700 t worldwide in 2001) used as flame-retardants for plastics and textiles. HBCDs exhibit typical properties of persistent organic pollutants (POPs). They are highly lipophilic and accumulate in biota. Increasing environmental concentrations of HBCDs, mostly reported as sum values, have been observed. As such, HBCDs have to be considered as potential emerging POPs, but their occurrence and environmental fate have not yet been addressed at the level of individual HBCD stereoisomers. Considering the six stereogenic centers of HBCDs, 16 stereoisomers, six diastereomeric pairs of enantiomers as well as four meso forms, can be deduced. Herein, we report spectroscopic and chromatographic data for eight out of 16 possible HBCD stereoisomers, which were isolated from a technical product. Six stereoisomers were identified as three pairs of enantiomers ((+/-) alpha-, beta-, and gamma-HBCDs), differing in optical rotation and chromatographic retention on a chiral phase. The crystal structures of these pairs of enantiomers were determined. Another two of these eight HBCD stereoisomers, not yet described in the literature, showed no optical rotation and are tentatively assigned as meso forms (delta- and epsilon-HBCD). The given spectroscopic and chromatographic information allows the unambiguous identification of eight HBCD stereoisomers and the occurrence, fate, and toxicology of these individual stereoisomers can now be studied

Heeb, NV; Schweizer, WB; Mattrel, P; et al. (2007) Solid-state conformations and absolute configurations of (+) and (-) alpha-, beta-, and gamma-hexabromocyclododecanes (HBCDs). Chemosphere (CHEMOSPHERE) 68:940-950.
Hexabromocyclododecanes (HBCDs) are high production volume chemicals used as flame retardants for plastics and textiles. They are currently produced in quantities exceeding 20 000 t/y. Despite this fact, the correct stereochemistry of most HBCDs is still not known. Six stereocenters are formed during bromination of cyclododecatrienes, resulting in mixtures of different stereoisomers. Considering all elements of symmetry, 16 different stereoisomers including six pairs of enantiomers as well as 4 meso forms are possible theoretically. Recently, we isolated 8 of the 16 possible stereoisomers from a technical HBCD mixture and assigned their relative configurations. Herein, we report on the isolation of 6 enantiomerically pure alpha-, beta-, and gamma-HBCDs, obtained from preparative chiral-phase liquid chromatography, and we present their absolute configurations, which were determined from X-ray diffraction analysis. The absolute configuration of (-) alpha-HBCD was found to be (1R,2R,5S,6R,9R,10S), while the one of (+) beta-HBCD is assigned to (1S,2S,5S,6R,9S,10R), whereas the one of (-) gamma-HBCD corresponds to (1S,2S,5S,6R,9R,10S). The given structural information allows the unambiguous identification of the six most important HBCD stereoisomers, which typically account for more than 95% of technical HBCDs. In addition, we compared the solid-state conformations of racemic and enantiomerically pure alpha-, beta-, and gamma-HBCDs. In all cases, vicinal dibromides adopted a synclinal (sc) conformation with torsion angles of 69 +/- 6degrees. A unique structural motive was common to all examined HBCD solid-state conformations. This conserved structure was described as an extended triple turn consisting of an arrangement of three pairs of synclinal and two antiperiplanar torsion angles.

Heeb, NV; Schweizer, WB; Mattrel, P; et al. (2007) Crystal structure analysis of enantiomerically pure (+) and (-) beta-hexabromocyclododecanes. Chemosphere 66(8):1590–1594.
The molecular structures of individual HBCD stereoisomers are not elucidated yet. Recently, we isolated 8 of the 16

possible stereoisomers from a technical HBCD mixture and tentatively assigned their relative configurations. Herein we report on the isolation of enantiomerically pure (+) and (-) beta-HBCDs, both obtained from preparative chiral-phase liquid chromatography, and we present their absolute configurations determined from X-ray diffraction analysis. The absolute configuration of (+) beta-HBCD was found to be (1S,2S,5S,6R,9S,10R), while the one of (-) beta-HBCD was assigned to (1R,2R,5R,6S,9R,10S). The given structural information allows, for the first time, the unambiguous identification of these two important HBCD stereoisomers, which are typically found in technical products at proportions of about 3-5% for each enantiomer

Chemical MFGS Association. (1997) Hexabromocyclododecane (HBCD): Determination of the vapor pressure using a spinning rotor gauge with cover letter dated 08/15/1997. EPA/OTS Doc #86970000839; NTIS No. OTS0573702.

Velsicol (Velsicol Chemical Corporation). (1990) Partition coefficient of dicamba, endrin vel 3510 and several industrial chemicals and flame retardants laboratory report with test data and cover letter. EPA/OTS Doc #86-900000269–900000269; NTIS No. OTS0523261.

Velsicol. (1990) Water solubility of several industrial chemicals flame retardants and a herbicide vel-3510 laboratory report with test data and cover letter. EPA/OTS Doc #86-900000270; NTIS No. OTS OTS0523262.

Wildlife International Limited. (1997) Determination of n-octanol/water partition coefficient with cover letter dated 06/27/1997. EPA/OTS Doc #86970000802 NTIS No. OTS0573665.

Wildlife International Limited. (1997) Hexabromocyclododecane (HBCD): Determination of water solubility with cover letter dated 06/27/1997. EPA/OTS Doc #86970000798; NTIS No. OTS073661.

3. TOXICOKINETICS

Yu CC and Atallah YH. (1980) Pharmacokinetics of HBCD in rats. pp 24. Vesichol Chemical Corporation (unpublished report).

3.1. ABSORPTION

Roper CS. (2005) The in vitro percutaneous absorption of radiolabelled hexabromocyclododecane (HBCD) through human skin. pp 36.

3.2. DISTRIBUTION

Arita, R: Miyazaki, K: Mure, S. (1983) Metabolic test of hexabromocyclododecane. Test on chemical substances used in household items. Studies on pharmacodynamics of hexabromocyclododecane. Department of Pharmacy, Hokkaido University Hospital (Unpublished report).

3.3. METABOLISM

Germer, S; Piersma, AH; van, d, V; et al. (2006) Subacute effects of the brominated flame retardants hexabromocyclododecane and tetrabromobisphenol A on hepatic cytochrome P450 levels in rats. Toxicology 218:229–236.
The brominated flame retardants tetrabromobisphenol A (TBBPA) and hexabromocyclododecane (HBCD) are found in the environment, e.g., in sediments and organisms, in food items, human blood samples and mother's milk. In this study, the effects of both compounds on rat hepatic cytochrome P450 (CYP) levels and activities were investigated. Juvenile/young male and female Wistar rats were treated orally with various doses via the feed (TBBPA) or by gavage (HBCD). After 28 days of treatment the animals were sacrificed and hepatic mRNA and

microsomes were isolated. HBCD treatment led to a significant induction of CYP2B1 mRNA, CYP2B1/2B2 protein and 7-pentoxyresorufin O-dependentylase (PROD) activity suggesting a phenobarbital-type of induction. Furthermore, a significant increase in CYP3A1/3A3 mRNA, CYP3A1 protein, and luciferin benzylether debenzylase (LBD) activity was found, being more pronounced in females than in males. The effect on CYP3A1/3A3 mRNA was significant in female rats at a daily dose of 3.0mg/kg body weight and above. HBCD exhibited no effects on CYP1A2 mRNA, CYP1A1/1A2 protein, or microsomal 7-ethoxyresorufin O-deethylase (EROD) activity suggesting lack of activation of the aryl hydrocarbon receptor. No significant effects on any of the parameters measured were obtained with TBBPA. Our findings suggest that oral exposure to HBCD induces drug-metabolising enzymes in rats probably via the CAR/PXR signalling pathway. Induction of CYPs and co-regulated enzymes of phase II of drug metabolism may affect homeostasis of endogenous substrates including steroid and thyroid hormones
This article may also be relevant to Sections 4.4 & 4.5.

Hakk, H; Letcher, RJ. (2003) Metabolism in the toxicokinetics and fate of brominated flame retardants--a review. Environ Int 29:801–828.
Several classes of brominated flame retardants (BFRs), namely polybrominated biphenyls (PBBs), polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A (TBBPA), hexabromocyclododecane (HBCCD), bis(2,4,6-tribromophenoxy)ethane (BTBPE), and tris(2,3-dibromopropyl)phosphate (Tris), have been identified as environmental contaminants. PBDEs, TBBPA, and HBCCD are of particular concern due to increasing environmental concentrations and their ubiquitous presence in the tissues of humans and wildlife from Europe, Japan, and North America. Regardless, the toxicokinetics, in particular metabolism, of BFRs has received little attention. The present review summarizes the current state of knowledge of BFR metabolism, which is an important factor in determining the bioaccumulation, fate, toxicokinetics, and potential toxicity of BFRs in exposed organisms. Of the minimal metabolism research done, BFRs have been shown to be susceptible to several metabolic processes including oxidative debromination, reductive debromination, oxidative CYP enzyme-mediated biotransformation, and/or Phase II conjugation (glucuronidation and sulfation).However, substantially more research on metabolism is necessary to fully assess BFR fate, uptake and elimination kinetics, metabolic pathways, inter-species differences, the influence of congener structure, and the potential health risks to exposed organisms

3.4. ELIMINATION

3.5. PHYSIOLOGICALLY BASED TOXICOKINETIC MODELS

4. HAZARD IDENTIFICATION

4.1. STUDIES IN HUMANS—EPIDEMIOLOGY, CASE REPORTS, CLINICAL CONTROLS

Brominated Chemicals. (2006) UK dietary intakes. Food survey information sheet 10/06. pp 31.

De Winter-Sorkina R, Bakker MI, Van Donkersgoed G and Van Klaveren JD. (2003) Dietary intake of brominated flame retardants by the Dutch population. pp 25. RIVM report 310305001, RIKILT repoer 2003.019, Bilthoven.

Fängström, B; Strid, A; Bergman, Å. (2005) Temporal trends of brominated flame retardants in milk from Stockholm mothers 1980-2004. pp 1-9. Department of Environmental Chemistry, University of Stockholm, Stockholm, Sweden. Available online at http://www.imm.ki.se/Datavard/PDF/mj%C3%B6lk_poolade_NV%20rapport%202005%20modersmjolk.pdf.

Haskell Labs (Haskell Laboratories). (1990) Letter from E.I Dupont de Nemours & Co to USEPA concerning enclosed studies on decabromodiphenyl ether, hexabromocyclododecane and 4-vinylcyclohexane with attachments (Sanitized). EPA/OTS Doc #86-900000119S; NTIS No. OTS0522190. . Skin irritation and sensitization tests with 20 human subjects exposed to 0.05 ml of 10% 1,2,5,6,9,10-hexabromocyclododecane led to no skin reactions at any examination.

Lignell, S; et al. (2003) Persistent organic pollutants (POP) in breast milk from primiparae women in Uppsala County, Sweden, 2002-2003. Report to the Swedish Environmental Protection Agency.

Lopez, D; Athasiadou, M; Athanassiadis, I; et al. (2004) A preliminary study of PBDEs and HBCDD in blood and milk from Mexican women. *In:* The third international workshop on brominated flame retardants BFR 2004. Book of abstracts. Edited by Alaee M et al. pp 483-487. http://www.bfr2004.com/Book%20of%20Abstracts.pdf.

Thomsen, C; Fróshaug, M; Leknes, H; Becher, G. (2003) Brominated flame retardants in breast milk from Norway. Organohalogen compounds 64.

Velsicol. (1978) Industrial hygiene survey, , el dorado, ark plant, fire master 680 unit and semi-works summary with attachments and cover letter dated 071978. EPA/OTS Doc #88-7800228; NTIS No. 0200544.

Weiss, J; Wallin, E; Axmon, A; et al. (2006) Hydroxy-PCBs, PBDEs, and HBCDDs in serum from an elderly population of Swedish fishermen's wives and associations with bone density. Environ Sci Technol 40:6282–6289. Lack of human exposure data is frequently reported as a critical gap in risk assessments of environmental pollutants, especially regarding "new" pollutants. The objectives of this study were to assess serum levels of the persistent 2,2',4,4',5,5'-hexachlorobiphenyl (CB-153), hydroxylated polychlorinated biphenyl metabolites (OH-PCBs), polybrominated diphenyl ethers (PBDEs), and hexabromocyclodode-canes (HBCDDs) in a group of Swedish middle-aged and elderly women expected to be relatively highly exposed, and to evaluate the impact of potential determinants (e.g., fish intake, age) for the inter-individual variation, as well as to investigate the association between these pollutants and bone density. No associations were found between bone mineral density or biochemical markers of bone metabolism and the analyzed environmental pollutants. Relatively high levels of CB-153 (median 260 ng/g fat) and sigma 3-OH-PCBs (median 1.7 ng/mL serum), and low concentrations of sigma6PBDEs (median 3.6 ng/g fat) were determined. Total level of HBCDDs in serum was quantified by gas chromatography with mass spectrometric detection (median 0.5 ng/g fat). HBCDD diastereomeric and enantiomeric patterns were determined by liquid chromatography with mass spectrometric detection. The dominating stereoisomer was (-)alpha-HBCDD, but 1-3% of gamma-HBCDD was also detected in the serum samples

Weiss, J; Meijer, L; Sauer, P; et al. (2004) PBDE and HBCDD levels in blood from Dutch mothers and infants—Analysis project within the Dutch Gronigen Infant Cohort. *In:* The third international workshop on brominated flame retardants- BFR Book of abstracts, edited by Alaee M, 200; pp. 71-74. http://www.bfr2004.com/Book%20of%20Abstracts.pdf.

WHO-GEMS/Food international dietary survey. (1998) Infant exposure to certain organochlorine contaminants from breast mil – A risk assessment. Food Safety Issues 98.4, WHO/FSF/FOS, Geneva, Switzerland.

4.2. LESS-THAN-LIFETIME AND CHRONIC STUDIES AND CANCER BIOASSAYS IN ANIMALS—ORAL AND INHALATION

Great Lakes Chemical Corporation. (1994) Initial submission: Letter from Great Lakes Chem Corp to dynamac corp/usepa submitting info re hexabromocyclododecane and bis(Tribromophenoxy) Ethane w/attchmts, dated 2/13/89. EPA/OTS Doc #FYI-OTS-0794-1055; NTIS No. OTS0001055.

GSRI (Gulf South Research Institute). (1994) Initial submission: Letter from Ethyl Corp to USEPA re technical and toxicity data on brominated flame retardants including hexabromocyclododecane, * w/attchmts, dated 5/23/88. EPA/OTS Doc #FYI-OTS-0794-0947, NTIS No. OTS0000947.

Acute Studies

BASF (BASF Corporation). (1990) Report on the study of the acute oral toxicity of hexabromocyclododecane in the mouse with cover letter dated 031290. EPA/OTS Doc #86-900000383; NTIS No. OTS0522946.

Chemical MFGS Association. (1997) Letter from Chem Mfgs Assoc to USEPA regarding: Toxicological

investigation of hexabromocyclododecane (Hbcd) With attachments, dated 06/27/1997. EPA/OTS Doc #FYI-OTS-1097-1306; NTIS No. OTS0001306.

Chemical MFGS Association. (1997) Letter from Chem Mfgs Assoc to USEPA regarding: Toxicity studies on hexabromocyclododecane, pentabromodiphenyl oxide, and octabromodiphenyl oxide with attachments, dated 11/26/1996. EPA/OTS Doc #FYI-OTS-0197-1281; NTIS No. OTS0001281.

IRDC (International Research and Development Corporation). (1978) Acute inhalation toxicity study in rats with hexabromocyclododecane with attachments and cover letter dated 042478. EPA/OTS Doc #88-7800137; NTIS No. 02000488.

IRDC. (1978) Acute toxicity studies in rabbits and rats with hexabromocyclododecane with attachments. EPA/OTS Doc #88-7800065; NTIS No. OTS02000051.

IRDC. (1978) Acute toxicity studies in rabbits and rats with residue of hexabromocyclododecane with attachments and cover letter dated 030178 EPA/OTS Doc #88-7800088; NTIS No. OTS0200466.

IRDC. (1990) Acute toxicity studies in rabbits and rats with test data and cover letter dated 03-08-90. EPA/OTS Doc #86-900000266: NTIS No. OTS0523258.

Lewis, AC; Palanker, AL. (1978a) A dermal LD50 study in albino rabbits and an inhalation LC50 study in albino rats. Test material GLS-S6-41A. Client: Saytech Inc. (not published). 78385-2, Consumer product testing.

Lewis, AC; Palanker, AL. (1978b)A primary dermal irritation study, a dermal corrosion study, and an ocular irritation study in albino rabbits and an oral LD50 study in albino rats. Test material GLS-S6-41A. Client: Saytech Inc. (not published). 78385-1, Consumer Product Testing.

Ogaswara, S; Fukushi, A; Midorikawa, Y. (1983) Report on acute toxicity test of pyroguard SR-103 in rats (not published).

Pharmakon Research International Inc. (1990) Acute exposure dermal toxicity test in rabbits (82 EPA/OECD) With attachments and cover letter dated 030890. EPA/OTC Doc #86-900000167; NTIS No. OTS0522238.

Pharmakon Research International Inc. (1990) Acute exposure oral toxicity study in rats (83 EPA/OECD) With attachments and cover letter dated 030890. EPA/OTS Doc #86-900000166; NTIS No. OTS0522237.

Tobe et al. (1984). Acute toxicity test of hexabromocyclododecane (not published). Research Center for Biological Safety, National Public Health Research Institute.

U.S. EPA. (1990d) Report on the study of the acute oral toxicity of hexabromocyclododecane in the mouse. Letter from BASF. 86-900000383, EPT/OTS Doc.

Wilson, PD; Leong, BKJ. (1977) Acute toxicity studies in rabbits and rats. Test material Firemaster 100 Lot 53 77.902. Sponsored by: Velsicol Chemical Corporation (not published). 163-499, International Research and Development Corporation.

Subchronic Studies

BASF. (1990) Hexabromocyclododecane: 28-day feeding trials with rats with test data and cover letter. EPA/OTS Doc #86-900000274; NTIS # OTS0523266.

BASF. (1990) Hexabromocyclododecane: 28-Day feeding trials with rats with cover letter dated 031290. EPA/OTS Doc #86-900000376; NTIS No. OTS0522939.

Zeller, H; Kirsch, P. (1969) Hexabromocyclododecane: 28-day feeding trials with rats. BASF (not published).

Chengelis, CP. (1997) A 28-day repeated dose oral toxicity study of HBCD in rats. pp 925. WIL Research Laboratories, Inc, Ashland, Ohio, USA.

Wil Research Laboratories Inc. (1997) A 28-day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 3/18/1997. EPA/OTS Doc #86970000747; NTIS No. OTS0558957.

Wil Research Laboratories Inc. (1997) A 28-day repeated dose oral toxicity study of hexabromocyclododecane (HBCD) in rats with cover letter dated 03/18/1997. EPA/OTS Doc #FYI-OTS-0397-1289, NTIS No. OTS0001289.

Wil Research Laboratories Inc. (1998) Addendum to final report (Thyroid histopathology), A 28-day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 6/18/1998. EPA/OTS Doc #86-980000155; NTIS No. OTS0559493.

Wil Research Laboratory Inc. (1998) Support: Report addendum, a 28-day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 6/19/1998 EPA/OTS Doc #FYI-OTS-0898-1289, NTIS No. OTS0001289.

Chengelis, CP. (2001) A 90-day oral (gavage) toxicity study of HBCD in rats. WIL-186012, pp 1527. Wil Reserach Laboratories, Inc, Ashland, Ohio, USA.

Chengelis, CP. (2002) Amendment to the final report: A 90-day oral (gavage) toxicity study of HBCD in rats. WIL-186012, pp 280. Wil Reserach Laboratories, Inc, Ashland, Ohio, USA.

Wil Research Laboratories Inc. (2002) Hexabromocyclododecane (HBCD): A 90-day oral (Gavage) Toxicity study of HBCD in r. EPA/OTS Doc #FYI-OTS-0102-001424; NTIS No. OTS0001424.

Wil Research Laboratories Inc. (2002) Support: Ltr fr Amer Chem Council to USEPA submitting 2 env effcts stdies on tetrabromobisphenol a & an addendum to a 90-day tox stdy on HBCD in rats, w/attchmnts & dtd 072502. EPA/OTS Doc #FYI-OTS-0902-1424; NTIS No. OTS0001424-1.

Pharmakologisches Institute. (1990) Hexabromocyclododecane: 90-Day feeding trials with rats with attachments and cover letter dated 031290. EPA/OTS Doc #86-900000380; NTIS No. OTS0522943.

Zeller, H; Kirsch, P. (1970) Hexabromocyclododecane: 90-day feeding trials with rats. BASF (not published).

Chronic Study

Kurakawa, Y; Inoue, T; Uchida, Y; Momma, J. (1984) Carcinogenesis test of flame retarder hexabromocyclododecane in mice. Albemarle Corporation. Department of Toxicology, National Public Health Research Institute, Biological Safety Test and Research Centre. (Unpublished report)

Unknown Exposure Duration

USEPA status report: Hexabromocyclododecane with attachments (2000) EPA/OTS Doc #8EHQ-0278-0065:0278–0065.

USEPA status report: Hexabromocyclododecane with attachments (2000) EPA/OTS Doc #8EHQ-0478-0137:0478–0137

4.3. REPRODUCTIVE/DEVELOPMENTAL STUDIES—ORAL AND INHALATION

Aneslmo, J; Cao, D; Karrison, T; et al. (2004) Fetal loss associated with excess thyroid hormone exposure JAMA 292:691-695.

Murai, T; Kawasaki, H; Kanoh, S. (1985) Studies on the toxicity of insecticides and food additives in pregnant rats. 7. Fetal toxicity of hexabromocyclododecane. Oyo Yakuri(Pharmacometrics) 29:981-986.

Schardein, JL. (1993) Food additives. In: Anonymous; eds. Chemically Induced Birth Defects. pp. 581–597.

Stump, DG. (1999) Prenatal developmental toxicity study of hexabromocyclododecane (HBCD) in rats. pp 410. WIL Research Laboratories, Inc, Ashland, Ohio, USA.

4.4. OTHER ENDPOINT-SPECIFIC STUDIES

Eriksson, P; Fisher, C; Wallin, M; et al. (2006) Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD). Environ Toxicol Pharmacol 21:317-322.
Brominated flame-retardants (BFRs) are a diverse group of global environmental pollutants. In the present study, we show that neonatal exposure to hexabromocyclododecane (HBCDD) can cause developmental behavioural defects that are similar to those recently reported for PBDEs and certain PCBs. Furthermore, HBCDD appears to be as potent as PBDEs in inducing developmental neurotoxic effects in mice. In this study, neonatal NMRI mouse pups were given either a single oral dose of 0.9 mg HBCDD/kg body weight, 13.5 mg HBCDD/kg body weight, or a 20% fat emulsion vehicle on postnatal day 10. At the age of 3 months, the mice were observed regarding spontaneous behaviour and concerning learning and memory capability. Mice exposed to 0.9 mg HBCDD or to 13.5 mg HBCDD/kg body weight showed a significantly altered spontaneous behaviour, manifested as a hyperactive condition and reduced habituation. Learning and memory, as observed in a Morris water maze, was also significantly affected in mice given the higher dose of HBCDD. (c) 2005 Elsevier B.V. All rights reserved

Eriksson, P; Viberg, H; Fischer, C; et al. (2002) A comparison on developmental neurotoxic effects of hexabromocyclododecan, 2,2´,4,4´,5,5´-hexabromodiphenyl ether (PBDE 153) and 2,2´,4,4´,5,5´-hexachlorobiphenyl (PCB 153). Abstract no 488. In DIOXIN 2002.

Eriksson P, Viberg H, Fischer M, et al. (2002) A comparison on developmental neurotoxic effects of hexabromocyclododecane, 2,2'4,4',5,5'-hexabromodiphenyl ether. Organohalogen Compounds 57:389-392.

Hale, RC; La Guardia, MJ; Harvey, E; et al. (2006) Brominated flame retardant concentrations and trends in abiotic media Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD). Chemosphere 64:181–186.

Lilienthal, H; van der Ven, L; Roth-Haerer, A; et al. (2006) Neurobehavioral toxicity of brominated flame retardants: Differential effects of PBDE-99, TBBPA and HBCD and endocrine relation. pp 3.

Magnusson, B; Kligman, AM. (1969) The identification of contact allergens by animal assay. The guinea pig maximization test. J Invest Dermatol 52:268-276.

Mariussen, E; Fonnum, F. (2003) The effect of brominated flame retardants on neurotransmitter uptake into rat brain synaptosomes and vesicles. Neurochem Int 43:533–542.
The environmental levels of brominated flame retardants (BFRs) are increasing, but little is known about their toxic effects. In this paper, we show that some of the most important BFRs in commercial use today, have a neurotoxicological potential. Hexabromocyclododecane (HBCD) and tetrabromobisphenol-A (TBBPA) inhibit plasma membrane uptake of the neurotransmitters dopamine, glutamate and gamma-amino-n-butyric acid (GABA) at a concentration level similar to what previously found for polychlorinated biphenyls (PCBs) and even for ecstasy. The IC(50) value for HBCD on dopamine uptake was 4 microM, and the IC(50) values for TBBPA were 9, 6 and 16 microM for dopamine, glutamate and GABA, respectively. HBCD also inhibited glutamate uptake at low concentrations, but never achieved more than 50% inhibition. The inhibition was primarily due to their effect on the membrane potential, measured by the membrane potential marker tetraphenylphosphonium bromide (TPP(+)). Other brominated flame retardants such as octaBDE and decaBDE did not have any effects on uptake. TBBPA, HBCD and even the pentabrominated diphenylether mixture (pentaBDE, DE-71, Great Lakes) also inhibited the vesicular uptake of dopamine with an IC(50) value of 3, 3 and 8 microM, respectively. The neurotoxicological consequences of these findings for environmental contaminants such as BFRs and PCBs are discussed

McDonnell (1972) " Human Patch Test." Haskell Laboratory Report. 185-72 Haskell Laboratory for Toxicology and Industrial Medicine, E I du Pont de Nemours and Company.

Microbiological Associates Inc. (1996) Hexabromocyclododecane (HBCD): Maximization test in guinea pigs with cover letter dated 12/12/1996. EPA/OTS Doc #86970000356; NTIS No. OTS0573550.

Momma, J; Kaniwa, M; Sekiguchi, H; et al. (1993) Dermatological evaluation of a flame retardant, hexabromocyclododecane (HBCD) on guinea pig by using the primary irritation, sensitization, phototoxicity and photosensitization of skin. Bull National Inst Hyg Sci 111:18-24.
As one of the projects in the safety evaluation of chemical constituents in common house-hold products, effects of hexabromocyclododecane (HBCD) were evaluated by primary skin irritation, skin sensitization, phototoxicity and photosensitization using guinea pigs. Primary skin irritation was not observed in HBCD emulsified in distilled water by the Draize test method. Skin sensitization test was carried out according to the maximization test method of Magnusson and Kligman. For this test, HBCD was dissolved in olive oil to give 5, 0.5 and 0.05%. When induction of sensitization occurred, challenged doses of 0.005, 0.05, 0.5 and 5% of HBCD (dissolved in acetone) were applied to its respective sensitized groups. The results showed that the induction dose of greater than 0.5% and the challenge dose of greater than 0.05% elicited a positive response. The increase in the concentration of induction and challenge doses did not further increase the percentage of positive response or the intensity of skin response. Phototoxicity test was carried out with 0, 0.5 and 5% of HBCD dissolved in acetone. Phototoxicity was not observed at all HBCD concentration tested. Photosensitization test was performed according to the Sato's adjuvant-strip method. The skin sensitization and challenge reaction doses were 5 and 0.5% and 0 and 0.5% HBCD (dissolved in acetone), respectively, and no positive reaction was observed. It is clear from the foregoing results that HBCD is a mild skin allergen

Nakamura, A; Momma, J; Sekiguchi, H; et al. (1994) A new protocol and criteria for quantitative determination of sensitization potencies of chemicals by guinea pig maximization test. Contact Dermatitis 31:72–85.
BIOSIS COPYRIGHT: BIOL ABS. This paper presents precise sensitization test data of 15 chemicals with a wide spectrum of sensitization potencies. and proposes a new protocol and criteria for quantitative evaluation of sensitization potencies of chemicals. The tests were performed according to the design of Magnusson and Kligman, changing the application concentrations for induction as well as for challenge phases. 3-dimensional relationships between mean response (or sensitization rate), induction and challenge concentrations were found in all chemicals tested. The following 2 values are proposed as a quantitative measure of sensitization potency: (a) the minimum induction concentration that induces a positive response; (b) the challenge concentration that induces a mean response approximately equal to 1.0 among the animals applied with the highest concentration for induction. Both values coincided with each other within the range of 1 order of magnitude in every compound except 2. The values varie

Schriks, M; Vrabie, CM; Gutleb, AC; et al. (2006b) T-screen to quantify functional potentiating, antagonistic and thyroid hormone-like activities of polyhalogenated aromatic hydrocarbons (PHAHs). Toxicol In Vitro 20:490-498.

van der Ven, LTM; Verhoef, A; van de Kuil, T; et al. (2006) A 28-day oral dose toxicity study enhanced to detect endocrine effects of hexabromocyclododecane in Wistar rats. Toxicol Sci 94:281-92. Epub 2006 Sep 19.
A 28-day repeated dose study in rats (OECD407) enhanced for endocrine and immune parameters was performed with hexabromocyclododecane (HBCD). Rats were exposed by daily gavage to HBCD dissolved in corn oil in 8 dose groups with doses ranging between 0 and 200 mg/kg bw per day (mkd). Evaluation consisted of dose-response analysis with calculation of a benchmark dose at the lower 95% one-sided confidence bound (BMDL) at predefined critical effect sizes (CESs) of 10-20%. The most remarkable findings were dose-related effects on the thyroid hormone axis, that is, decreased total thyroxin (TT4, BMDL 55.5 mkd at CES--10%), increased pituitary weight (29 mkd at 10%) and increased immunostaining of TSH in the pituitary, increased thyroid weight (1.6 mkd at 10%), and thyroid follicle cell activation. These effects were restricted to females. Female rats also showed increased absolute liver weights (22.9 mkd at 20%) and induction of T4-glucuronyl transferase (4.1 mkd at 10%), suggesting that aberrant metabolization of T4 triggers feedback activation of the thyroid hormone system. These effects were accompanied by possibly secondary effects, including increased cholesterol (7.4 mkd at 10%), increased tibial bone mineral density (> 49 mkd at 10%), both in females, and decreased splenocyte counts (0.3-6.3 mkd at 20%; only evaluated in males). Overall, female rats appeared to be more sensitive to HBCD than male rats, and an overall

BMDL is proposed at 1.6 mkd, based on a 10% increase of the thyroid weight, which was the most sensitive parameter in the sequence of events

Wolhiser, MR; Anderson, PK. (2003) Hexabromocyclododecane: contact sensitization potential via the local lymph node assay(including a primary irritancy screen) using CBA/J mice. Study ID 031013, pp 24. Toxicology & Environmental Rearch and Consulting, The Dow Chemical Company, Midland, Michigan, USA.

4.5. MECHANISTIC DATA AND OTHER STUDIES IN SUPPORT OF THE MODE OF ACTION

Canton, RF; Sanderson, JT; Nijmeijer, S; et al. (2006) In vitro effects of brominated flame retardants and metabolites on CYP17 catalytic activity: a novel mechanism of action? Toxicol Appl Pharmacol 216:274–281.
Fire incidents have decreased significantly over the last 20 years due, in part, to regulations requiring addition of flame retardants (FRs) to consumer products. Five major classes of brominated flame retardants (BFRs) are hexabromocyclododecane isomers (HBCDs), tetrabromobisphenol-A (TBBPA) and three commercial mixtures of penta-, octa- and deca-polybrominated diphenyl ether (PBDE) congeners, which are used extensively as commercial FR additives. Furthermore, concentrations of PBDEs have been rapidly increasing during the 1999s in human breast milk and a number of endocrine effects have been reported. We used the H295R human adrenocortical carcinoma cell line to assess possible effects of some of these BFRs (PBDEs and several of their hydroxylated (OH) and methoxylated (CH(3)O) metabolites or analogues), TBBPA and brominated phenols (BPs) on the combined 17alpha-hydroxylase and 17,20-lyase activities of CYP17. CYP17 enzyme catalyzes an important step in sex steroidogenesis and is responsible for the biosynthesis of dehydroepiandrosterone (DHEA) and androstenedione in the adrenals. In order to study possible interactions with BFRs, a novel enzymatic method was developed. The precursor substrate of CYP17, pregnenolone, was added to control and exposed H295R cells, and enzymatic production of DHEA was measured using a radioimmunoassay. In order to avoid pregnenolone metabolism via different pathways, specific chemical inhibitor compounds were used. None of the parent/precursor BFRs had a significant effect ($P < 0.05$) on CYP17 activity except for BDE-183, which showed significant inhibition of CYP17 activity at the highest concentration tested (10 muM), with no signs of cytotoxicity as measured by mitochondrial toxicity tests (MTT). A strong inhibition of CYP17 activity was found for 6-OH-2,2',4,4'-tetrabromoDE (6-OH-BDE47) with a concentration-dependent decrease of almost 90% at 10 muM, but with a concurrent decrease in cell viability at the higher concentrations. Replacement of the 6-OH group by a 6-CH(3)O group eliminated this cytotoxic effect, but CYP17 activity measured as DHEA production was still significantly inhibited. Other OH- or CH(3)O-PBDE analogues were used to elucidate possible structural properties behind this CYP17 inhibition and associated cytotoxicity, but no distinct structure activity relationship could be determined. These in vitro results indicate that OH and CH(3)O-PBDEs have potential to interfere with CYP17 activity for which the in vivo relevance still has to be adequately determined

Harju, M; Hamers, T; Kamstra, JH; et al. (2007) Quantitative structure-activity relationship modeling on in vitro endocrine effects and metabolic stability involving 26 selected brominated flame retardants. Environ Toxicol Chem 26:816–826.
In this work, quantitative structure-activity relationships (QSARs) were developed to aid human and environmental risk assessment processes for brominated flame retardants (BFRs). Brominated flame retardants, such as the high-production-volume chemicals polybrominated diphenyl ethers (PBDEs), tetrabromobisphenol A, and hexabromocyclododecane, have been identified as potential endocrine disruptors. Quantitative structure-activity relationship models were built based on the in vitro potencies of 26 selected BFRs. The in vitro assays included interactions with, for example, androgen, progesterone, estrogen, and dioxin (aryl hydrocarbon) receptor, plus competition with thyroxine for its plasma carrier protein (transthyretin), inhibition of estradiol sulfation via sulfotransferase, and finally, rate of metabolization. The QSAR modeling, a number of physicochemical parameters were calculated describing the electronic, lipophilic, and structural characteristics of the molecules. These include frontier molecular orbitals, molecular charges, polarities, log octanol/water partitioning coefficient, and two- and three-dimensional molecularproperties. Experimental properties were included and measured for PBDEs, such as their individual ultraviolet spectra (200-320 nm) and retention times on three different high-performance liquid chromatography columns and one nonpolar gas chromatography column. Quantitative structure-activity relationship models based on androgen antagonism and metabolic degradation rates generally gave similar results, suggesting

that lower-brominated PBDEs with bromine substitutions in ortho positions and bromine-free meta- and para positions had the highest potencies and metabolic degradation rates. Predictions made for the constituents of the technical flame retardant Bromkal 70-5DE found BDE 17 to be a potent androgen antagonist and BDE 66, which is a relevant PBDE in environmental samples, to be only a weak antagonist

Hamers, T; Kamstra, JH; Sonneveld, E; et al. (2006) In vitro profiling of the endocrine-disrupting potency of brominated flame retardants. Toxicol Sci 92:157–173.
Over the last few years, increasing evidence has become available that some brominated flame retardants (BFRs) may have endocrine-disrupting (ED) potencies. The goal of the current study was to perform a systematic in vitro screening of the ED potencies of BFRs (1) to elucidate possible modes of action of BFRs in man and wildlife and (2) to classify BFRs with similar profiles of ED potencies. A test set of 27 individual BFRs were selected, consisting of 19 polybrominated diphenyl ether congeners, tetrabromobisphenol-A, hexabromocyclododecane, 2,4,6-tribromophenol, ortho-hydroxylated brominated diphenyl ether 47, and tetrabromobisphenol-A-bis(2,3)dibromopropyl ether. All BFRs were tested for their potency to interact with the arylhydrocarbon receptor, androgen receptor (AR), progesterone receptor (PR), and estrogen receptor. In addition, all BFRs were tested for their potency to inhibit estradiol (sulfation by estradiol sulfotransferase (E2SULT), to interfere with thyroid hormone 3,3',5-triiodothyronine (T3)-mediated cell proliferation, and to compete with T3-precursor thyroxine for binding to the plasma transport protein transthyretin (TTR). The results of the in vitro screening indicated that BFRs have ED potencies, some of which had not or only marginally been described before (AR antagonism, PR antagonism, E2SULT inhibition, and potentiation of T3-mediated effects). For some BFRs, the potency to induce AR antagonism, E2SULT inhibition, and TTR competition was higher than for natural ligands or clinical drugs used as positive controls. Based on their similarity in ED profiles, BFRs were classified into five different clusters. These findings support further investigation of the potential ED effects of these environmentally relevant BFRs in man and wildlife

Reistad, T; Fonnum, F; Mariussen, E. (2006) Neurotoxicity of the pentabrominated diphenyl ether mixture, DE-71, and hexabromocyclododecane (HBCD) in rat cerebellar granule cells in vitro. Arch Toxicol 80:785–796.
Polybrominated diphenyl ethers (PBDE) and hexabromocyclododecane (HBCD) are compounds used as additive flame retardants in plastics, electronic equipment, and textiles. The aim of the present study was to investigate the in vitro effects of the pentabrominated diphenyl ether mixture, DE-71, and HBCD on cerebellar granule cells (CGC). Both DE-71 and HBCD induced death of CGC in low micromolar concentrations. The NMDA receptor antagonist MK801 (3 muM), and the antioxidant alpha-tocopherol (50 muM) significantly reduced the cell death. Incubation of the compounds together with the rat liver post-mitochondrial (S9) fraction reduced cell death by 58 and 64% for DE-71 and HBCD, respectively. No ROS formation and no elevation in intracellular calcium were observed. We further demonstrated apoptotic morphology (Hoechst straining) after exposure to low levels of the two brominated flame retardants and signs of DNA laddering were found after DE-71 exposure. However, other hallmarks of apoptosis, like caspase activity, were absent indicating an atypical form of apoptosis induced by DE-71. After intraperitoneal injection of the two compounds both DE-71 and HBCD were found in significant amounts in brain (559 +/- 194 and 49 +/- 13 mug/kg, respectively) and liver (4,010 +/- 2,437 and 1,248 +/- 505 mug/kg, respectively) 72 h after injection. Our results indicate that the lower brominated PBDEs have a higher potency of bioaccumulation than HBCD, and that both compounds have a neurotoxic potential in vitro

Yamada-Okabe, T; Sakai, H; Kashima, Y; et al. (2005) Modulation at a cellular level of the thyroid hormone receptor-mediated gene expression by 1,2,5,6,9,10-hexabromocyclododecane (HBCD), 4,4'-diiodobiphenyl (DIB), and nitrofen (NIP). Toxicol Lett 155:127–133.
Previously, we demonstrated that some endocrine disrupting chemicals affected thyroid hormone receptor (TR)-mediated gene expression in HeLaTR cells that stably expressed the human TRalpha1. To examine whether widely used brominated flame retardants and pesticides affect TR-mediated gene expression, those with organohalogen, which is also present in T3, were screened. To monitor the TR-mediated gene expression, HeLaTR cells were transfected with a luciferase gene that was linked to the thyroid hormone responsive element. Thus, transcription of the luciferase gene in HeLaTR cells is driven by TR. By screening 38 chemical agents, it was found that 4,4'-diiodobiphenyl (DIB), markedly, and 1,2,5,6,9,10-hexabromocyclododecane (HBCD) and nitrofen (NIP), to a much lesser extent but significantly, enhanced the expression of the luciferase gene at concentrations that did not affect the growth of HeLaTR cells. DIB also augmented the E2-induced expression of the luciferase gene that was linked to the estrogen responsive element in MCF7 cells, whereas HBCD and NIP did not. These results indicate that DIB augments TR- and ER-mediated gene expression, but HBCD and NIP affect only TR-mediated gene expression.

Thus, there is a potential risk that HBCD, DIB, and NIP act as endocrine disrupters in animals and human beings.

Genotoxicity

Microbial Assays

ANONYMOUS. (1983) Salmonella mutagenesis test results. NTP Tech Bull (9):5-6,1983 :TECH–6.

BASF. (1990) Ames test with hexabromides with cover letter dated 031290. EPA/OTS Doc #86-900000379; NTIS No. OTS0522942.
1,2,5,6,9,10-Hexabromocyclododecane was evaluated for mutagenicity in vitro in the TA98, TA100, and TA1537 strains of Salmonella typhimurium, both in the presence and absence of Arochlor-induced rat liver S-9 metabolic activation. Bacterial cultures without metabolic activation were tested at concentrations of 0.0, 31.5, 100, 315, 1000, or 3000 ug/plate; bacterial cultures with metabolic activation were tested at 0, 3.15, 10, 31.5, 100, 315, 1000, and 3000 ug/plate; the test compound formed a precipitate at concentrations greater than 1000 ug/plate, possibly affecting the accuracy of the test. Some aromatic and olefinic compounds are metabolized to mutagenic epoxides; epoxides can be inactivated by epoxide hydratase or by conjugation to glutathione. A second trial was performed in TA98 with the same concentrations of test compound and with the addition of 1,1,1-trichloropropene 2,3-oxide, an inhibitor of epoxide hydratase and glutathione, to increase the sensitivity of the test towards compounds activated to mutagenic epoxides. The treatment did not increase the frequency of histidine revertants, either with or without metabolic activation, indicating that the test compound was negative for mutagenicity in Salmonella under the conditions of this assay. Positive control treatment with 10 ug/plate benzo(a)pyrene, 10 ug/plate 2-aminoanthracene, or 90 ug/plate

BASF. (1990) Ames metabolic activation test to assess the potential mutagenic effect of und no. 49 With cover letter dated 031290. EPA/OTS Doc #86-900000385; NTIS No. OTS0522948.
1,2,5,6,9,10-Hexabromocyclododecane was evaluated for mutagenicity in vitro in the TA98, TA100, and TA1535 strains of Salmonella typhimurium, both in the presence and absence of Arochlor-induced rat liver S-9 metabolic activation. Bacterial cultures with and without metabolic activation were tested at concentrations of 0, 10, 100, 1000, and 10000 ug/plate; the test compound formed a precipitate at 10000 ug/plate, possibly affecting the accuracy of the test. The treatment did not increase the frequency of histidine revertants, either with or without metabolic activation, indicating that the test compound was not mutagenic in Salmonella under the conditions of this assay. Positive control treatment with 10 ug/plate

Baskin, AD; Phillips, BM. (1977) Mutagenicity of two lots of FM-100, Lot 53 and residue of lot 3322 in the absence and presence of metabolic activation. Sponsored by: Velsicol Chemical Corporation (not published). Industrial BIO TEST Laboratories, Inc.

Ethyl Corporation. (1990) Genetic toxicology salmonella/microsomal assay on hexabromocyclododecane with cover letter dated 030890. EPA/OTS Doc #86-900000164; NTIS No. 0522235.
Hexabromocyclododecane was evaluated for mutagenicity by plate assay in the bacterium, Salmonella typhimurium, strains TA1535, TA1537, TA98, and TA100, both in the presence and absence of Aroclor 1254-induced rat liver homogenate. Positive (substance not identified), solvent (acetone), and untreated controls were utilized. Test concentrations ranged from 0.05 to 5.0 mg/plate. The high dose did not produce toxicity in any strain. A dose-related increase was observed in the number of revertant colonies in strains TA100 and TA1535 both in the presence and absence of metabolic activation

Great Lakes Chemical Corporation. (1990) In vitro microbiological mutagenicity studies of four CIBA-Geigy corporation compounds (Final report) With test data and cover letter. EPA/OTS Doc #86-900000262; NTIS No. OTS0523254.

GSRI. (1979) Mutagenicity test of GLS-S6-41A. (not published). Gulf South Research Institute.

Hossack, DJN; Richold, M; Jones, E; Bellamy, RP. (1978) Ames metabolic activation test to assess the potential

mutagenic effect of compound no. 49. 1978; pp 1-2. Huntingdon Research Centre.

Indust Bio-Test Labs. (1990) Mutagenicity of two lots of fm-100 lot 53 and residue of lot 3322 in the absence and presence of metabolic activation with test data and cover letter. EPA/OTS Doc #86-900000267; NTIS No. OTS0523259.

Litton Bionetics Inc. (1990) Mutagenicity evaluation of 421-32b (Final report) With test data and cover letter. Submitted under TSCA Section 8D; EPA Document No. 86-900000265; NTIS No. OTS0523257. Hexabromocyclododecane (Compound 421-32B) was evaluated for mutagenicity by plate assay in two microorganisms, Saccharomyces cerevisiae, strain D4, and Salmonella typhimurium, strains TA1535, TA1537, TA1538, TA98, and TA100, both in the presence and absence of Aroclor 1254-induced rat liver homogenate. Positive (MNNG, NF, QM, ANTH, AAF, AMQ, and DMNA) and solvent (DMSO) controls were utilized. Test concentrations ranged from 0.5 to 50 ug/plate; the low dose was less than that which demonstrated any toxic effect, and the high dose produced quantitative or qualitative evidence of some chemically-induced physiological effects. Ogaswara, S; Hanafusa, T. (1993) Report on mutagenicity test on pyroguard SR-103 using microorganisms(not published).

Simmon, VF; Poole, DC; Newell, GW; Skinner, WA. (1976) In vitro microbiological mutagenicity studies of four CIBA-GEIGY corporation compounds. Prepared for CIBA-Geigy Corporation (not published). 5702, SRI Project LSC.

Zeiger, E; Anderson, B; Haworth, S; et al. (1987) Salmonella mutagenicity tests. 3.Results from the testing of 255 chemicals. Environ Mutagen 9(Suppl 9):1-110.

DNA Repair Test

Ethyl Corporation. (1990) Genetic toxicology rat hepatocyte primary culture/DNA repair test on hexabromocyclododecane with cover letter dated 030890 EPA Doc #86-900000163; NTIS No. OTS0522234. Genotoxicity of HBCD Bottoms (hexabromocyclodecane) was evaluated in vitro in the rat hepatocyte primary culture/DNA repair assay. The compound was tested at concentrations of 0.05, 0.1, 0.5, 1, 5, 10, 50, 100, 500, 1000 ug/well, in 2 ml of acetone. The negative control and positive control groups were cell cultures exposed to acetone only, and acetone plus 2-acetylaminofluorene (1x10E-6 M), respectively. The treatment was cytotoxic to the cell cultures at 1000 ug/well. A dose-dependent increase in the rate of DNA repair was observed at dose levels of 5, 10, 50, 100, 500, or 1000 ug/well, indicating that hexabromocyclodecane was positive for genotoxicity in this cell culture assay

Chromosome Aberrations

Gudi, R; Schadly, EH. (1996) Chromosome aberrations in human peripheral blood lymphocytes. (not published). Chemical Manufacturers Association.

Microbiological Associates (1996) Hexabromocyclododecane (HBCD): Chromosome aberrations in human peripheral blood lymphocytes with cover letter dated 12/12/1996 EPA/OTS Doc #86970000358; NTIS No. OTS0573552.

Micronucleus Test

Engelhardt, Hoffman. (2000) Cytogenetic study in vivo with of hexabromocyclododecane in the mouse micronucleus test after two intraperitoneal administrations. BASF, Ludwigshafen, Germany.

Recombination Test

Helleday, T; Tuominen, KL; Bergman, A; et al. (1999) Brominated flame retardants induce intragenic recombination in mammalian cells. Mutat Res 439(2):137–147.
In the present study we have examined the effects of brominated flame retardants (BFR) and several other

environmental contaminants in two in vitro assays for intragenic recombination at an endogenous locus in mammalian cells. A total ten compounds were investigated, i. e., two technical PCB mixtures (Aroclor 1221 and Aroclor 1254), DDT, PCP, tetrabromobisphenol A (TBBPA), 4,4'-bischlorophenyl sulfone (BCPS), hexabromocyclododecane (HBCD) and the three different polybrominated diphenylethers (PBDEs): 2-bromodiphenylether (MBDE), 3,4-dibromodiphenylether (DBDE) and 2,4,2', 4'-tetrabromodiphenylether (TBDE). In the SPD8 assay system statistically significant increases in recombination frequency were observed with Aroclor 1221, BCPS, DBDE, DDT, HBCD, MBDE and TBDE. In the Sp5 assay system, only DBDE, HBCD and MBDE caused statistically significant increases in recombination frequency. In conclusion, our findings indicate that the modern additives to plastic, i.e., HBCD and PBDEs, as well as the plastic monomer BCPS may have the same effect to human health as DDT and PCBs, in terms of inducing genetic recombination, which is known to provoke a number of diseases, including cancer