Priority Existing Chemical Assessment Report No. 34

Australian Government
Department of Health and Ageing
NICNAS

Hexabromocyclododecane

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NATIONAL INDUSTRIAL CHEMICALS NOTIFICATION AND ASSESSMENT SCHEME
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Priority Existing Chemical Assessment Report Hexabromocyclododecane - June 2012

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Preface

This assessment was carried out under the National Industrial Chemicals Notification and Assessment Scheme (NICNAS) that was established by the *Industrial Chemicals (Notification and Assessment) Act 1989* (Cth) (the Act), which came into operation on 17 July 1990.

The principal aim of NICNAS is to aid in the protection of people at work, the public and the environment from the harmful effects of industrial chemicals.

NICNAS assessments are carried out in conjunction with the Australian Government Department of Sustainability, Environment, Water, Population and Communities, which carries out the environmental assessment for NICNAS.

NICNAS has two major assessment programs: the assessment of human health and safety and environmental effects of new industrial chemicals prior to importation or manufacture; and the other focusing on the assessment of chemicals already in use in Australia, in response to specific concerns about their health and/or environmental effects.

There is an established mechanism within NICNAS for prioritising and assessing the many thousands of existing chemicals in use in Australia. Chemicals selected for assessment are referred to as Priority Existing Chemicals.

This Priority Existing Chemical report has been prepared by the Director of NICNAS, in accordance with the Act. Under the Act, manufacturers and importers of Priority Existing Chemicals are required to apply for assessment. Applicants for assessment are given a draft copy of the report and 28 days to advise the Director of any errors. Following the correction of any errors, the Director provides applicants and other interested parties with a copy of the draft assessment report for consideration. This is a period of public comment lasting for 28 days, during which requests for variation of the report may be made. Where variations are requested, the Director's decision concerning each request is made available to each respondent and to other interested parties (for a further period of 28 days). Notices in relation to public comment and decisions made appear in the Commonwealth *Chemical Gazette*.

In accordance with the Act, publication of this report revokes the declaration of this chemical as a Priority Existing Chemical; therefore, manufacturers and importers wishing to introduce this chemical in the future need not apply for assessment. However, manufacturers and importers need to be aware of their duty to provide any new information to NICNAS, as required under s. 64 of the Act.

Copies of this and other Priority Existing Chemical reports are available on the NICNAS website. Hard copies are available free of charge from NICNAS from the following address:

GPO Box 58, Sydney, NSW 2001, AUSTRALIA
Tel: +61 2 8577 8800
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Other information about NICNAS (also available on request and on the NICNAS website) includes:

- NICNAS Service Charter
- information sheets on NICNAS Company Registration
- information sheets on the Priority Existing Chemicals and New Chemical assessment programs
- safety information sheets on chemicals that have been assessed as Priority Existing Chemicals
- details for the NICNAS Handbook for Notifiers
- details for the Commonwealth *Chemical Gazette*.

More information on NICNAS can be found at the NICNAS website:
http://www.nicnas.gov.au

Other information on the management of workplace chemicals can be found at the website of Safe Work Australia:
http://www.safeworkaustralia.gov.au

Overview

Background

Hexabromocyclododecane (HBCD) CAS No. 25637-99-4 was declared a Priority Existing Chemical (PEC) for a full risk assessment under the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) by notice in the Commonwealth *Chemical Gazette* of 7 June 2005.

HBCD is one of a number of polybrominated flame retardants (PBFRs). In 2001, a preliminary PEC assessment of PBFRs as a group was conducted by NICNAS, as there was concern over the widespread use of flame retardants in household and industrial situations, and a report focusing on occupational, public health and environmental exposure to PBFRs was published. The report recommended that a full risk assessment be conducted once testing of these chemicals was completed internationally.

A follow-up survey conducted by NICNAS in October 2004 indicated a significant increase in the use of HBCD in Australia compared to the period 1998–99. It was determined that a full risk assessment of HBCD as a PEC would allow assessment of its potential occupational, public health and environmental risks and formulation of appropriate recommendations for the safe use of the chemical.

Manufacture and importation

HBCD is not manufactured in Australia. It is imported into Australia as liquid dispersions, in expandable and extruded polystyrene (EPS) resin and as a component of the plastic in finished articles. Finished articles containing HBCD include extruded polystyrene (XPS) boards, office equipment such as inkjet printers, projectors, scanners and ventilation units for offices. The assessment showed that there was a decrease in the import of HBCD over the years, with 91 tonnes imported in 2005–06 and approximately 60.5 tonnes in 2009–10. It was reported in 2011 that technical grade HBCD (powder or granules) is no longer being imported into Australia since 2010.

Uses

HBCD is an additive flame retardant, meaning that it is incorporated into the polymer matrix but does not chemically bind to it. HBCD is used in the EPS resin form in domestic and industrial building insulation, packaging of industrial products and beanbag fills. HBCD is also used in XPS boards in domestic and industrial insulation and in automotive upholstery. Other uses are as a polypropylene resin in housing for domestic electrical appliances and as a textile coating additive in blinds, public seating and garments. A small amount of raw HBCD is used in the manufacture of flame retarded polystyrene masterbatch, which is used in an injection moulding process in the manufacture of ceiling fan covers.

Health effects

Limited data are available on the toxicokinetics and metabolism of HBCD. Studies in rats indicate that HBCD is rapidly absorbed from the gastrointestinal tract with a half-life of approximately 2 h. HBCD is widely distributed in the body, with the highest concentrations found in adipose tissue and muscles, followed by the liver, lung, kidney, blood and brain. In animal experiments, excretion was also rapid, with majority of the radiolabelled HBCD excreted in the faeces as metabolites or non-extractable radioactivity within 72 h. Only a small percentage was detected in the urine. Three metabolites were extracted from the faeces of rats following administration of radiolabelled HBCD; however, the identity of the metabolites was not known.

HBCD has low acute oral, dermal and inhalation toxicity. It is not an eye or skin irritant in animals. It is not a skin sensitiser in animals or humans. There are no data available on the potential for respiratory sensitisation.

Repeat dose toxicity studies consistently revealed dose-dependent increases in liver weights in 28 d and 90 d studies in both sexes; in a 90 d study, up to a 48% increase in liver weight in female rats was reported at the highest dose. The liver weight changes were reported to be reversible except at the highest dose tested. Histopathological changes were minimal and only reported in the 90 d studies. The increase in liver weight was likely to be due to enzyme induction and an adaptation response; however, the magnitude of increase and its persistence after recovery period indicate that the increase in liver weight could be considered an adverse effect.

Thyroid effects were also noted in female rats. Relative thyroid weights were increased by 33% in mid- and high-dose females. There was minimal thyroid follicular cell hypertrophy. Serum concentrations of thyroid hormone (T4) were dose-dependently decreased at all doses in males and females. Increased pituitary weights were also noted in female rats. These changes in liver, thyroid hormone and pituitary could possibly be explained by enzyme induction in the liver, leading to increased excretion of thyroid hormone and stimulation, by feedback mechanism, of pituitary and thyroid. T4-uridine-diphosphate glucoronyl transferase in the liver has been shown to be induced in rats (both sexes) treated with HBCD.

Based on increase in liver weights, a no observable adverse effect level (NOAEL) of 10 mg/kg bw/d was established for repeated dose toxicity.

HBCD was not genotoxic in any of the animal tests and did not show carcinogenic properties in a chronic study in mice.

In reproductive toxicity studies in rats, HBCD did not show any marked adverse effects on fertility parameters. In a 2-generation reproductive study, no significant changes were noted in copulation index, gestation index or the number of implantations. Reduced primordial follicles were noted in mid- and high-dose F1 females; however, they were within the range of historical control values from 4 earlier studies performed in the same laboratory that performed the current study. Moreover, the number of primordial follicles was the same at the mid and high doses, with no dose response relationship. There were also large variations within the groups. No significant difference was seen between control and HBCD-treated groups in the incidence of clinical signs of toxicity in either male or female F0 and F1 rats during the test period.

The 28 d repeat-dose study reported inhibited oogenesis and reduced number of follicles, with sparse ripening follicles in the ovaries at the highest dose of 4700 mg/kg bw/d. No effects were noted in male rats exposed to the same high dose, except for small inner sexual organs. Prostate weights were increased in a 90 d oral study in rats. The effects seen in male and female rats are considered to be mediated through the endocrine system.

A clear NOAEL for effects on fertility could not be established in any of the reproductive studies. However, based on the reduced fertility index (not statistically significant) and reduced primordial follicles in the F1 generation rats in the two-generation reproductive study, a NOAEL of 10.2 mg/kg bw/d was considered.

Developmental effects in rats were seen in two reproductive toxicity studies. In a one-generation study, bodyweights of F1 pups were decreased in a dose-dependent manner. The time to vaginal opening was delayed in the female pups at the top dose. Testes weights were decreased at low doses, with a significant dose-response relationship. Systemic effects at high doses of HBCD were decreased kidney and thymus weights in both sexes and decreased adrenals, heart, brain and prostate weights in F1 males. A decrease in bone mineral density in females and decreased total mineral content, total area and cortical thickness in males in the femur and tibial bones were also noted. Both male and female pups showed marked dose-dependent decreases in liver apolar retinoid levels.

In a 2-generation reproductive toxicity study, a dose-dependent increase in pup mortality during lactation was observed in the F2 generation (35% at the high dose and ~15% at the mid dose) in the absence of clear clinical signs of toxicity in the dams. Decrease in bodyweights of male F1 and F2 pups and female F2 pups were also noted at the high dose. In the F2 generation, the incidence of pups showing complete eye opening was lowered compared to controls. Delayed development in F2 pups was indicated by the reduced incidence of male and female pups showing eye opening on postnatal day (PND) 14. The development of basic reflexes was also affected at the highest dose, leading to shorter time response in the surface righting reflex in F1 male pups on PND 5 at the higher dose level. Other effects noted in F2 weanlings were decreased weights of kidney, brain, spleen, adrenal, epididymis, ventral prostate and ovary at mid and high doses.

There were also indications of developmental neurotoxicity in a study in adult mice that were exposed to HBCD as pups. However, this study was not performed according to OECD guidelines.

Based on low bodyweights of pups and high mortality during lactation at the mid and high dose in the 2-generation reproductive study, HBCD was considered to be a developmental toxic chemical. A NOAEL of 10.2 mg/kg bw/d was established for developmental toxicity of HBCD. HBCD also has the potential to cause harm when transferred through lactation.

Selecting a NOAEL for risk characterisation from developmental studies would estimate risk only for a small section of the population (females of child-bearing age) and thus a NOAEL based on adverse effects observed in both male and female animals should also be used for estimating risk to the general population. The other pronounced effect of HBCD in animal studies was increase in liver weight in both male and female animals. The NOAEL for this effect in a reliable and well-conducted 28 d oral study was 10 mg/kg bw/d, which is very similar to the NOAEL from the reproductive study. Therefore the risk calculated using this NOAEL would also cover risk from repeated exposure in the occupational use situation as well as to the general population, especially since HBCD is known to be persistent.

Public exposure and health risk

HBCD is used in consumer articles as an additive flame retardant. Consumers using the treated products may be exposed to HBCD that diffuses out of the articles. Exposure to HBCD from treated articles is expected to be mostly through the dermal route. Exposure through inhalation or ingestion of treated products is considered negligible.

A potential source of dermal exposure to HBCD is automotive upholstery treated with HBCD. Estimates of dermal exposure from this source, however, indicated very low exposure and therefore low risk to adults as well as children.

Indirect exposure to HBCD through the environment may occur by consumption of food and drinking water contaminated by HBCD and by inhalation of indoor and outdoor air. Exposure from inhalation was estimated to be very low, resulting in low risk of adverse effects by this route. On the basis of limited available information, exposure to HBCD from dietary sources appears to be very low.

Exposure to HBCD could also occur through ingestion or inhalation of dust/soil, especially in children. Indoor dust may contain HBCD released from HBCD-containing articles in the house. International data of concentrations of HBCD in household dust showed great variability. Estimation of exposure via this route showed that toddlers have the highest exposure; however, the risk of developing adverse health effects was quite low. Australian data on HBCD

concentrations in breast milk are not available. Oral exposure of infants to HBCD was estimated using HBCD levels in human breast milk reported in a UK study. The risk to infants was estimated to be low through exposure to HBCD in breast milk.

Occupational exposure and health risk

The extent of occupational exposure to HBCD depends on the form of HBCD used – powder or granular forms or aqueous solution – the nature of the work and the different use patterns. Exposure to workers handling HBCD was estimated based on exposures during importation and packaging, in the polymer industry, in the textile industry and while handling HBCD-containing products/articles.

A NOAEL for exposure via the inhalation route was not available to determine the inhalation risk to workers. However, since HBCD administered orally was determined to be completely absorbed (100% absorption), the NOAEL from an oral study was used to estimate risk from inhalation of HBCD during occupational handling. For determining risk from dermal exposure, modelled dermal exposure data were converted to internal dose using the dermal absorption values for powder, granules and liquid formulations. Total exposure was determined as the sum of the internal dose estimated from dermal exposure values and dermal absorption rate and inhalation exposure assuming 100% absorption from respiratory tract. In the occupational exposure assessment the median and the 90th percentile values are used to represent the typical and reasonable worst-case estimates, respectively.

The risk of harmful effects by inhalation to workers handling the powder or granular formulations during importation and transport is likely to be negligible, except in the event of an accident or spill. Workers in the polymer industry may be exposed during weighing HBCD, compounding, conversion or moulding activities. Both the powder and the granular forms have been used in compounding in Australia. The risk to workers of acute adverse health effects such as inhalation toxicity, skin, eye and respiratory irritation and skin sensitisation is low. However, the risk of chronic harmful effects from exposure during weighing of HBCD powder is high. Risk to workers is low during weighing of the granular form and compounding in the typical scenarios. However, in the realistic worst-case scenarios for all of these processes, margins of exposures were considerably lower than 100, indicating high risk to workers using powdered as well as granular HBCD.

Although powder formulations are currently not used in Australia, risks from powder formulations were estimated because these formulations could be imported and used in future, if not regulated.

Risk to workers handling semi-finished and end use products is low, as these products contain HBCD at very low concentrations and the HBCD is either incorporated into a plastic matrix or fixed onto fibres. Workers handling polystyrene foam products containing HBCD during activities such as cutting, sawing and machining to manufacture shaped products can also be exposed to HBCD. However, the concentration of the chemicals in these products is very low, and the risk of adverse health effects from inhalation or dermal exposure is not expected to be of concern.

Environmental effects

For many of the aquatic toxicity tests conducted, the EC_{50}/LC_{50} values could not be identified. Several tests measured endpoints in terms of total HBCD and, based on solubility, it appears that, for all trophic levels, the no observed effect concentration (NOEC) for HBCD in acute aquatic toxicity tests is at or around the measured total HBCD solubility of 3.4 µg/L.

The lowest EC_{50} was for the marine diatom *S. costatum*, with a geometric mean EC_{50} of10.5 µg/L. The lowest chronic toxicity indices were for *Daphnia magna*, with a NOEC of 3.1 µg/L and a maximum acceptable toxic concentration (MATC) of 4.2 µg/L. Based on these data, HBCD is very toxic to aquatic organisms.

For terrestrial ecotoxicity, test results for plants (seedling emergence study only) showed no effects due to HBCD exposure up to a measured soil level of 6200 mg/kg. The earthworm reproduction study produced an EC_{50} of 771 mg/kg soil. A NOEC of 128 mg/kg was also established in this study, even though a 15% inhibition of reproduction (compared with the controls) was still observed at the lowest tested mean measured concentration of 51.5 mg/kg. An extrapolated EC_{10} of 21.6 mg/kg was calculated for this study.

HBCD is not specifically listed in the Australian Dangerous Goods (ADG) Code. Based on its environmental toxicity, HBCD falls under Class 9, Packaging group III and UN number 3077 (environmentally hazardous substance).

Environmental exposure

The majority of HBCD (>95%) is used to produce flame retardant EPS resins and the environment is unlikely to be directly exposed, except during disposal of the resins. As a result of product manufacturing and textile treatment, the amount of HBCD released to air, water and solid waste is estimated to be 242, 609 and 44 kg, respectively, per annum.

There is considerable uncertainty regarding the fate of HBCD in the environment. While laboratory data indicate that HBCD degrades faster under anaerobic conditions than aerobic conditions, the mechanisms for this are unclear. Monitoring data from sediments in the environment (where conditions are most likely to be anaerobic) show a wide range of HBCD levels and do not indicate anaerobic degradation of HBCD, suggesting persistence in the environment is much longer than that shown in laboratory studies. Further, the detections in biota and abiotic samples in remote regions provide additional evidence of the persistent nature of HBCD in the environment.

There is also uncertainty about the isomer composition of HBCD found in the environment. While the γ isomer is dominant in commercial formulations, the α isomer is dominant in higher trophic level animals. This can be explained by bioisomerisation (formation of the α-HBCD from γ-HBCD in certain animals) or there may be a preference for uptake of the α isomer. Further, the α isomer is approximately 20 times more soluble than the γ isomer, so, where HBCD is present in the water column, α-HBCD would be expected to dominate.

HBCD is very bioaccumulative, with bioconcentration factors (total HBCD in whole fish) of between 8800 and 13 000. In addition, HBCD levels in biota support a conclusion that the substance bioaccumulates and biomagnifies through the food chain.

Overall, the available evidence indicates that HBCD is persistent and very bioaccumulative in the environment.

Environmental risks

Calculating 'safe' concentrations for compounds such as HBCD that are pesistent in the environment, are bioaccumulative and also biomagnify in the food chain is difficult because potential adverse effects may not become evident for very long periods of time – much longer than can be captured by standard toxicity testing.

A risk quotient method that compares toxicity to environmental exposure was used to estimate risk. Using derived predicted no effect concentrations (PNECs) for water, sediment and soil, and comparing these to exposure estimates for different exposure scenarios, predicted environmental concentration (PEC)/PNEC ratios >1 were identified for a number of scenarios. For surface water and sediment, this occurred when there were local releases from industries manufacturing end-use products with resins containing HBCD, or where HBCD is used in manufacturing end-use textiles. Similarly, for the terrestrial compartment, this occurred when there were local releases (both processing and end-use product manufacturing operations) from agricultural soils treated with biosolids, or from soils that are irrigated using effluent from treatment plants. The risk to aquatic species arising from use of HBCD in plastic or textile industry is low, as indicated by risk quotients (RQ) of <1. However, the sediment RQ for most use scenarios of HBCD indicated that HBCD concentrations in the Australian sediments have the potential to cause adverse effects. A potential local risk is also determined for terrestrial organisms from levels in soils amended with biosolids. However, this risk subsided to acceptable level for soils irrigated with effluent for up to 10 years.

Calculation of a risk quotient in air is not possible; however, it must be considered that the presence of HBCD in the atmosphere warrants concern in light of strong evidence that the substance is persistent and has the potential to travel long distances.

Overall, as a result of certain exposure scenarios, there is sufficient evidence that HBCD is persistent and bioaccumulative in the environment. In addition, it is very toxic to aquatic organisms. HBCD is considered to meet the Persistent Organic Pollutants (POPs) criteria for persistence, bioaccumulation and toxicity listed under the Stockholm Convention on Persistant Organic Pollutants (the Stockholm Convention). Because of these characteristics, the risks posed by this chemical cannot be fully quantified using standard methodology, and a cautious risk management approach may be necessary.

Recommendations

This section provides recommendations arising from the assessment of HBCD. Recommendations are directed principally at regulatory bodies and importers and formulators of HBCD and HBCD products. Implicit in these recommendations is that best practice is implemented to minimise occupational and public exposure and environmental impact.

HBCD is persistent, bioaccumulative and toxic, especially to aquatic organisms. Furthermore, it is not only considered to meet the POPs criteria as prescribed under the Stockholm Convention but also monitoring data for persistence, and laboratory and field data for bioaccumulation and bio-POPmagnification, indicate that the chemical substantially exceeds the criteria. In addition, while the POPs criteria do not prescribe numerical values for toxicity, there are ecotoxicity data indicating the potential for damage to the environment. The predicted half-life in air is marginally greater than 2 days, and monitoring data show evidence of long-range atmospheric transport of HBCD.

In animal studies, HBCD showed adverse effects in repeat-dose and developmental toxicity studies, although effects in repeat dose and reproductive toxicity studies were not significant enough to warrant classification based on these effects. There may be health concerns based on the effects on liver and thyroid and indications of developmental and transient neurobehavioral effects. Exposure assessment indicates that there is potential for workers handling HBCD to be exposed to levels that will pose a risk of adverse health effects. Public exposure to HBCD in the environment resulting from release of HBCD into household dust is not of concern due to the estimated low-level exposure.

The assessment indicates that the greatest risk is to the environment and workers handling HBCD, and this needs to be managed.

Occupational health and safety

Recommendation 1 (to Safe Work Australia)

Classification

Based on the assessment of the available hazard data and in accordance with the *Approved criteria for classifying hazardous substances* (NOHSC, 2004), HBCD is determined to be hazardous and is classified as:

- R63 Possible risk of harm to the unborn child (Toxic to reproduction, Category 3).
- R64 May cause harm to breastfed babies.

It is recommended that this classification for HBCD be included in the Hazardous Substances Information System (HSIS) as soon as possible.

The appropriate risk phrases for mixtures containing HBCD are as follows:

Risk Phrase	Concentration Cut-off
R63	≥5%
R64	≥1%

The following safety phrases are also recommended for HBCD:

- S22 Do not breathe dust.
- S60 This material and its container must be disposed of as hazardous waste.
- S61 Avoid release to the environment. Refer to special instructions/Material Safety Data Sheets.

The classification of HBCD under the Globally Harmonised System (GHS) of Classification and Labelling is provided in Appendix 1 along with the signal words and hazard statements and is as follows:

Health Hazards

- Toxic to reproduction Category 2

Environmental Hazards

- Acute toxicity Category 1
- Chronic toxicity Category 1

Recommendation 2. Hazard communication (to Industry)

MSDS and label amendments

It is recommended that importers and employers take note of the hazard classification, and amend Material Safety Data Sheets (MSDS), labels and training material, paying particular attention to the following points:

- inclusion of the health hazards, risk and safety phrases as contained in Recommendation 1
- correct information on the concentration cut-offs for mixtures containing HBCD as provided in Recommendation 1.
- that according to the ADG code, based on its environmental toxicity, HBCD falls under Class 9. Packaging group III and UN number 3077 (environmentally hazardous substance**Error! Bookmark not defined.**).

Recommendation 3 (to industry)

Based on the assessment findings, powdered or granulated HBCD should be handled under local exhaust ventilation. Workers should wear face masks, gloves and overalls to reduce exposure to HBCD.

Recommendation 4 (to industry)

Given the risks identified from the use of HBCD, importers of HBCD and manufacturers and importers of HBCD containing products and articles should move away from the import and use of HBCD, HBCD products and articles containing HBCD, in applications where safer alternatives and technologies are commercially available to support the objectives of Recommendation 6.

Recommendation 5 (to government): Compliance with State and Territory legislation

It is recommended that the state and territory occupational health and safety authorities review uptake of the new information in the MSDS and labels, and the safety measures recommended in this assessment.

Environmental safety

Recommendation 6 (to the Standing Council for Environment and Water (SCEW).

It is recommended that the SCEW develop an Action Plan to address the currently unacceptable risk of HBCD levels in the Australian environment arising from production and use of products and articles containing HBCD, taking account of:

- The risk assessment of HBCD finds unacceptable risks to the environment and to workers involved in certain tasks with HBCD.
- HBCD has been subject to an assessment by the Technical Committee of the Stockholm Convention on Persistent Organic Pollutants and HBCD has been found to meet the criteria for a Persistent Organic Pollutant (POP) under the Stockholm Convention. It is currently under discussion for listing in the Stockholm Convention.
- Commercially available, safer chemical alternatives and technologies cannot be used for all applications of HBCD.

The Action Plan should constitute a national approach involving federal, state and territory agencies and should address the full life cycle of HBCD in Australia as a chemical entity, in products and in articles.

Secondary notification

Under s. 64 of the *Industrial Chemicals (Notification and Assessment) Act 1989*, the secondary notification of a chemical that has been assessed under the Act may be required where change of any circumstances that may warrant a reassessment of its hazards and risks occurs.

In the case of HBCD, specific circumstances include the following:

Additional information has become available to the introducers of HBCD that is relevant to the adverse health or environmental risks associated with HBCD.

The Director of NICNAS must be notified within 28 days of the introducer becoming aware of the above or other circumstances prescribed under s. 64(2) of the Act. It is an offence under s. 64 of the Act if the Director is not notified of the change in circumstances specified above.

Acronyms and abbreviations

ADG CODE	Australian Code for the Transport of Dangerous Goods by Road and Rail
AICS	Australian Inventory of Chemical Substances
BAF	bioaccumulation factor
BCF	bioconcentration factor
BFR	brominated flame retardant
BMF	biomagnification factor
BSEF	Bromine Science and Environmental Forum
bw	bodyweight
CAS	Chemical Abstracts Service
CFR	Code of Federal Regulations (US)
CHO	Chinese hamster ovary
CSF	Chemicals Stakeholder Forum
CTD	characteristic travel distance
d	day
DecaBDE	decabromodiphenyl ether
DEFRA	Department for Environment Food and Rural Affairs
DEH	Australian Government Department of the Environment and Heritage[1]
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DPM	disintegration per minute
EC	European Commission
EC_{50}	median effective concentration
EPS	expandable polystyrene
ESR	Existing Substances regulation
EU	European Union
FR	flame retardant
FORS	Federal Office of Road Safety
FSANZ	Food Standards Australia and New Zealand
g	gram

[1] Now the Australian Government Department of Sustainability, Environment, Water, Population and Communities (DSEWPaC).

GC/MS	gas chromatography/mass spectrometry
GHS	Globally Harmonized System of Classification and Labelling of Chemicals
GLP	Good Laboratory Practice
h	hour
HBCD	hexabromocyclododecane
HIPS	high impact polystyrene
HMEM	Hank's minimal essential medium
hPa	hectopascal
HPLC	high-performance liquid chromatography
HPBL	human peripheral blood lymphocytes
HPVC	high production volume chemical
IUCLID	International Uniform Chemical Information Database
iv	intravenous
kg	kilogram
K_{ow}	octanol/water partition coefficient
L	litre
LC_{50}	median lethal concentration
LD_{50}	median lethal dose
LEV	local exhaust ventilation
LC/MS	liquid chromatography/mass spectrometry
LLNA	local lymph node assay
LOAEL	lowest-observed-adverse-effect level
LOD	limit of detection
LOQ	limit of quantitation
lw	lipid weight
LRTAP	Long-Range Transboundary Air Pollution
MATC	Maximum Acceptable Toxic Concentration
m^3	cubic metre
mg	milligram
mg/cm^3	milligrams per cubic centimetre
mg/kg bw	milligrams per kilogram bodyweight
mg/kg bw/d	mg/kg bodyweight/day
min	minute
mL	millilitre
µg	microgram
MSDS	Material Safety Data Sheet

NADPH	Nicotininamide adenine dinucleotide phosphate
ng	nanogram
ng/g lw	nanogram/gram lipid weight
NOAEL	no-observed-adverse-effect level
NOEC	no-observed-effect concentration
NOHSC	National Occupational Health and Safety Commission
OECD	Organisation for Economic Cooperation and Development
OECD TG	OECD Test Guideline
OPPTS	Office of Prevention, Pesticides and Toxic Substances (US)
OSPAR	Oslo–Paris Convention For The Protection Of The Marine Environment Of The North-East Atlantic
Pa	pascals
PBDE	polybrominated diphenyl ether
PBFR	polybrominated flame-retardant
PBT	persistent, bioaccumulative and toxic
PEC	priority existing chemical, predicted environmental concentration
PND	postnatal day
PNEC	predicted no effect concentration
POP	persistent organic pollutant
ppb	parts per billion
PPE	personal protective equipment
ppm	parts per million
PS	polystyrene
RIA	radioimmunoassay
SD	standard deviation
SI	stimulation index
SIAP	SIDS Initial Assessment Profile
SIDS	Screening Information Data Set
STP	sewage treatment plants
TBBPA	tetrabromobisphenol A
TSCA	Toxic Substances Control Act (US EPA)
TGA	Therapeutic Goods Administration
TWA	time weighted average
US EPA	United States Environmental Protection Agency
XPS	extruded polystyrene

1. Introduction

1.1 Declaration

Hexabromocyclododecane (HBCD), CAS No. 25637-99-4 was declared a Priority Existing Chemical (PEC) for a full risk assessment under the *Industrial Chemicals (Notification and Assessment) Act 1989* (the Act) by notice in the *Chemical Gazette* of 7 June 2005.

The basis for declaration as a PEC was that:

- HBCD is used as a flame retardant in polystyrene foam and in polystyrene beads that are used in insulation of articles such as baby car seats. It is also used in canvas products for industrial and domestic use, other styrene resins, latex binders, unsaturated polyesters and textile coatings.
- Adverse effects from exposure to HBCD, such as increase in liver weights and thyroid-related hyperplasia have been reported. Emerging evidence indicates that HBCD is persistent in the atmosphere.

In 2001, NICNAS conducted a preliminary PEC assessment to determine the extent of use and use pattern of the PBFRs, and published a report that contained an assessment of the occupational and public and environmental exposure to PBFRs. The report recommended that a full risk assessment be conducted once testing of these chemicals internationally was completed.

1.2 Objectives

The objectives of this assessment are to:

- identify the extent of use of HBCD in Australia
- assess the health and environmental hazards associated with HBCD
- estimate the potential public, occupational and environmental exposure to HBCD in Australia
- evaluate the potential risk of adverse effects to the general public, workers and the environment
- make recommendations to minimise public and occupational health risks and environmental risks.

1.3 Sources of information

Literature review

Information for the assessment was obtained from published literature sources, from the EU Risk Assessment Report on HBCD (EU RAR, 2008), and from the International Uniform Chemical Information Database (IUCLID) for HBCD. Various international databases were accessed to obtain toxicological and exposure information on HBCD and a comprehensive search of internet sources was undertaken.

Information from these reviews was supplemented with relevant studies from literature surveys conducted up to December 2010. After this date, new data were identified by

regular search alerts on HBCD through PubMed and ScienceDirect database systems. Reports, up to June 2011, with significant new information were included in this assessment.

In this report, references not marked with an asterisk have been reviewed for the purposes of this assessment. References not examined but quoted from the key reviews as secondary citations are also included in this assessment and marked with an asterisk.

Industry

In accordance with the *Industrial Chemicals (Notification and Assessment) Act 1989*, manufacturers and importers of HBCD, and those wishing to manufacture and import HBCD while the chemical was a PEC, were required to apply for assessment and supply information. Importers of HBCD as raw material or formulated products and articles were required to provide data to NICNAS on the import and use of HBCD between July 2003 and June 2006 for assessment. Seven of the 12 applicants also provided overseas and local MSDSs for the technical raw mixture, expandable polystyrene (EPS) and some products. Applicants also provided information on the import and use of articles containing HBCD. The use and importation volumes were updated to 2010 through information provided by industry.

1.4 Peer review

The report has been subjected to internal peer review by NICNAS during all stages of preparation. Human health hazard sections were also reviewed by an external expert, Dr Peter Abbott, currently at Bioscience Consulting.

1.5 Applicants

Amtrade International Pty Ltd
6th Floor, 574, St Kilda Road
Melbourne VIC 3004

Brava Australia
60 Mary Street
St Peters NSW 2044

Chemcolour Industries Aust. Pty Ltd
19-25 Anne Street
St Marys NSW 2760

Office of Environment and Heritage
P.O. Box A290
Sydney South NSW 1232

Huntsman Chemical Company Aust. Pty Ltd
Gate 3, 765 Ballarat Road
Deer Park; VIC 3023

Maleplas International Pty Ltd
P.O. Box 511
Mentone VIC 3194

Plastral Pty Ltd
P.O. Box 94
Matraville NSW 2036

Prime EPS Pty Ltd
29 Roslyn Road
Belmont VIC 3216

Clariant (Australia) Pty Ltd
675 Warrigal Road
Chadstone VIC 3148

Dow Chemicals (Aust) Ltd
541–583 Kororoit Creek Road
Altona VIC 3018

Epson Australia Pty Ltd
3 Talavera Road
Macquarie Park NSW 2113

Hunter Douglas Ltd
338 Victoria Road
Rydalmere NSW 2116

International Sales & Marketing (ISM)
262 Highett Road
Highett VIC 3190

Marchem Australasia Pty Ltd
P.O. Box 242
North Braybrook VIC 3019

BASF Australia Ltd
Level 12, 28 Freshwater Place
Southbank VIC 3006

2. Background

2.1 Flame retardants

Flame retardants are chemicals that inhibit or reduce the spread of fire when added to combustible material such as clothes and plastic casings. There are more than 175 different types of flame retardants, which are generally divided into classes that include the halogenated organic (usually brominated or chlorinated), phosphorus-containing, nitrogen-containing, and inorganic flame retardants. The brominated flame retardants (BFRs) represent a major industry, involving high-production chemicals with a wide variety of uses, mainly because of their low cost and high performance efficiency.

The basic mechanisms of flame retardancy vary depending on the specific flame retardant and substrate. Flame retardants can be additive or reactive. Additive flame retardants are added to a polymer without bonding or reacting with the polymer. For instance, they could be mixed into plastics before, during, or after polymerisation and dispersed evenly throughout the product but not chemically bound to it.

As they are not chemically bonded, additive flame retardants sometimes tend to bleed out of a product and vaporise or collect at the surface – a process known as "blooming", resulting in the gradual loss of flame retardancy. The degree (i.e. rate) at which blooming may occur is dependent on a number of factors, which include size and shape of the flame retarding molecule/polymer, geometric structure of the plastic matrix and stability of the flame retarding molecule/compound.

Laundering of materials treated with additive flame retardants can result in gradual leaching, and flame retardants applied as surface coatings can also be displaced through physical wear and tear of the coating over time.

In contrast with additive flame retardants, reactive flame retardants undergo reactions that chemically bind them to the raw materials that are used in the final product. This prevents them from bleeding out of the polymer, resulting in the product's retention of its flame retardant property.

The choice of a given flame retardant depends on the type of application, and their suitability is subject to variables such as the material to be flame-retarded, the fire safety standards with which the product must comply, cost considerations and recyclability. For base chemicals that are being flame-retarded, the effects on the chemical properties of the end product, such as tensility, flexibility and elongation, as well as the effects on the product during mixing and transformation, e.g. properties of the polymer melt, need to be considered. For additive flame retardants, compatibility with the polymer or the textile being treated is important as it avoids their migration to the surface. Migration of flame retardants to the surface of polymer or articles (blooming) results in the reduction of the permanency of the flame retardant property of the products. Exposure considerations at each life cycle stage of the flame-retarded chemical, such as during production and transport of raw materials, manufacturing, assembling of semi-finished products, use of end products and service life and waste disposal, recycling or incineration, all need to be taken into account (OECD, 1994). HBCD is used solely as an additive flame retardant. HBCD, being an additive flame retardant, is not chemically bonded into the polymer structure, but is physically bound within the polymer matrix.

Mechanism of action of brominated flame retardants

Solid materials burn when a heat source increases the internal kinetic activity of molecules in the material and they start to decompose into shorter chain molecules that eventually vaporise into gases (pyrolysis). These gases react with oxygen (O_2) in the air and burn. Heat from this combustion causes further pyrolysis and further combustion in an ongoing cycle. Highly reactive free radicals (e.g. $H^{\cdot}$, $^{\cdot}OH$, $R^{\cdot}$, $O^{\cdot\cdot}$ and $^{\cdot}OR$) are released during pyrolysis and help sustain a fire by propagating a radical chain reaction, releasing large quantities of energy into the flames.

Flame retardants can act in various ways to interfere with combustion during a particular stage of this process, e.g. during heating, decomposition, ignition or flame spread. They can act to decrease the ignition capacity by increasing the capacity of the product to withstand heat or, once a fire has already begun, they can reduce the tendency of the fire to spread by reacting with the product and forming a less flammable char or non-combustible gaseous layer.

Brominated flame retardants mainly act by hindering the ignition or combustion process through interfering with the free radical mechanism in the gaseous phase of the combustion process. When brominated flame retardant compounds absorb enough heat, they release bromine (Br·) as a free radical. Br· free radicals are heavy and low-energy radicals, and they react with the flammable gaseous hydrocarbon molecules to give HBr. The HBr then reacts with the high-energy H· and ·OH radicals to give water and the much lower energy Br· radicals, which are then available to begin a new cycle of H· and ·OH radical removal. The reaction of the bromine with the reactive radicals interferes with the propagation of chain reactions and withdraws energy from the combustion–propagation mechanism. The exothermic processes are stopped and the system cools down, and the supply of flammable gases is reduced or suppressed. The effectiveness of halogenated flame retardants depends on the quantity of the halogen atoms they contain (e.g. 10 bromine atoms in 1 molecule of decaBDE) and also, very strongly, on the control of the halogen release. For instance, because chlorine (another halogen) is released over a wider range of temperatures than bromine, it is then present in the flame zone at lower concentrations and so is less effective. Bromine is released over a narrow temperature range, thus resulting in optimal concentrations in the flame zone.

2.2 International perspective

HBCD has attracted attention as a chemical of concern not only within academia but also at a regional and global level by national authorities and international bodies such as the European Union (EU) and the United Nations (UN). HBCD and other flame retardants began to be subjects of concern in the 1980s and 1990s when several environmental monitoring programs conducted primarily in Europe and North America revealed that increasing levels of some fire retardants were detected in air, sewage sludge, sediment, fish, aquatic birds, marine mammals and other wildlife.

The increasing interest in HBCD resulted in a number of international studies and risk characterisations of this chemical.

2.2.1 European Union

In the European Union (EU), HBCD was identified as a Substance of Very High Concern (SVHC) under Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH), meeting the criteria of a persistent, bioaccumulative and toxic (PBT) substance pursuant to Art. 57(d) in the *European Community Regulation on*

Chemicals and their Safe Use (Regulation No. 1907/2006) (REACH regulation). In February 2011, HBCD was moved from the candidate list to the authorisation list, known as Annex XIV, under the EU's REACH regulation, which provides that PBT substances be subject to authorisation. Substances in Annex XIV cannot be placed on the market or used unless authorisation has been granted for a specific use.

On 8 December 2010, the Committee for Risk Assessment (RAC) of the European Chemicals Agency (ECHA) adopted the harmonised classification and labelling of HBCD as follows:

Classification: Repr. 2 – H361 (Suspected of damaging fertility or the unborn child)

Lact. – H362 (May cause harm to breastfed children)

Specific concentration limits: None

Labelling: GHS08 (pictogram depicting 'Health Hazard'), Wng (Warning), H361, H362**7.390**

Prior to consideration under REACH, HBCD had undergone an EU risk assessment for environment and human health under the EU *Existing Substances Regulation* 793/93/EEC (EURAR, 2008a). The Swedish Government was assigned as the lead (rapporteur). The EU Council Regulation provides the framework for the evaluation and control of the risk of existing substances. Under this framework, member States prepared Risk Assessment Reports on priority substances. The draft EU Risk Assessment Report (EURAR) prepared by Sweden was supported by the Technical Committee on New and Existing Substances (TC-NES) and presented to the European Commission Scientific Committee on Health and Environmental Risks (SCHER) for review. SCHER gave its opinion on both human health and environmental parts of the report (SCHER, 2008a & 2008b). Based on the information available then, SCHER concluded that the bioaccumulation (B) and toxicity (T) criteria in the PBT assessment were satisfied, while the persistence (P) was on the borderline. The Swedish Chemicals Agency (2007) also reviewed the available information on alternative substances and technologies of HBCD in *Strategy for limiting risks: hexabromocyclododecane (HBCCD)* (Swedish Chemical Agency, 2007).

In the EURAR (2008), HBCD was considered toxic to the environment (T-substance) largely because of the 21 d no observed effect concentration (NOEC) of 3.1 µg/L in daphnia (EC criterion for NOEC <10 µg/L). The EURAR indicated an absence of persistence based on degradation half-lives of 1.5 and 7 d for anaerobic sediments and soils, respectively; however, HBCD was considered to be persistent (P) because it could be found in biota of remote regions such as the Arctic.

The EURAR conclusions identified no risk to consumers. Furthermore, no risk was found for workers when standard industrial hygiene measures are applied (current EU practice). However, the TC-NES adopted the view that HBCD was a PBT chemical, the main concern being an increase of environmental concentrations in past years. TC-NES also concluded that a complete dataset was available and it was unlikely that new data would need to be generated for registration under REACH. Several specific risks to the environment were identified and a classification of HBCD as R50/53 was also recommended.

Based on the health effects and in accordance with Directive 67/548/EEC, HBCD was classified and labelled as follows:

Classification: Repr. Cat 3;

R63 (Possible risk of harm to the unborn child)

R64 (May cause harm to breastfed babies)

Specific concentration limits: None

Labelling: Xn; R 63 - 64;

S36/37-53 (Wear suitable protective clothing and gloves – Avoid exposure – obtain special instructions before use

The Nordic Council of Ministers prepared a report (TemaNord, 2008) in which information on environmental properties for HBCD was compared with the criteria for POP categorization. In the TemaNord report (2008), HBCD was classified as "P" based on temperature-adjusted degradation half-lives of 2 isomers of HBCD in aerobic sediments (12 ^{o}C) and negligible degradation in soil from one particular study (Davis et al., 2004). The TemaNord document was used to recommend adding HBCD to Annex A of the United Nations Stockholm Convention on POPs and Annex 1 of the UNECE protocol on POPs under Long-Range Transboundary Air Pollution (LRTAP).

Monitoring and assessment of pollution in the Arctic by the Arctic Council's Arctic Monitoring and Assessment Programme (AMAP) has shown HBCD as one of the pollutants of the Arctic (de Wit et al., 2010).

A report prepared for the European Chemicals Agency (ECHA looked at potential alternatives to HBCD (ECHA, 2008). The report concluded that, although there are a number of alternative flame retardants available, none is suitable to replace HBCD in its main end uses i.e. extruded polystyrene (XPS) or expandable polystyrene (EPS) for insulation, as the required loadings of alternative flame retardants impair the structure and properties of the finished product to the extent that it is no longer suitable for use. Other forms of insulation in place of EPS and XPS could be used, but they could be less appropriate for some specific use scenarios or may incorporate different environmental issues, such as increased energy costs during transportation.

Alternative fire retardants are available to replace HBCD in high impact polystyrenes (HIPS) but they are all required to be used at considerably higher loadings. Similarly, there are a wide range of different flame retardant formulations in textile coatings, although it is uncertain whether the human health and environmental impacts of these alternatives are any less than those associated with HBCD products. Some of the alternative fire retardants listed in the report are:

For HIPS: antimony trioxide (ATO), decabromodiphenyl ether/ATO, decabromodiphenylethane/ATO, ethylenebis-(tetrabromophthalimide)/ATO, resorcinol bis(biphenyl phosphate) and Bis phenol A bis (biphenyl phosphate)

For textiles: decabromodiphenyl ether, chlorinated paraffins, ammonium polyphosphates

For EPS/XPS: phenolic foam, polyurethane and polyisocyanurate products

Alternative insulations such as thermal barriers, loose-fill insulation and blanket insulation (incorporating glass wool, rock wool and gypsum board).

Health and environmental effects of these alternative flame retardants are discussed in the report.

2.2.2 UNEP Stockholm Convention on Persistent Organic Pollutants

In 2008 Norway submitted a proposal to the UNEP Stockholm Convention for listing HBCD in Annex A of the Convention pursuant to para. 1 of Art. 8 of the Convention. To support the proposal, the Nordic Council of Ministers prepared a report on HBCD in relation to the screening criteria of the Stockholm Convention, entitled *Hexabromocyclododecane as a Possible Global POP* (TemaNord, 2008).

At the fifth meeting of the POPs Review Committee (POPRC5) held in October 2009, the Committee reviewed the information on HBCD and concluded that HBCD met the criteria for adverse effects, persistence, bioaccumulation and long-range transport and agreed to the preparation of a risk profile.

The risk profile concluded that HBCD is sufficiently persistent to be of global concern, has a strong potential to bioaccumulate and biomagnify and is very toxic to aquatic organisms. In mammals, studies have shown reproductive, developmental and behavioral effects. HBCD has potential to interfere with the hypothalamic-pituitary-thyroid (HPT) axis and disrupt normal development. At environmentally relevant doses HBCD could be toxic to aquatic organisms and egg production in birds. 8.340

Based on the available evidence, the risk profile concluded that HBCD is likely, as a result of long-range environmental transport, to lead to significant adverse environmental and/or human health effects, such that global action is warranted.

The draft risk profile for HBCD was evaluated and adopted by the POPs Review Committee at its sixth meeting in October 2010 (POPRC6), the Committee decided that, "in accordance with paragraph 7 (a) of Article 8 of the Convention, HBCD is likely, as a result of its long range environmental transport, to lead to significant adverse human health and environmental effects such that global action is warranted". The Committee also decided to establish an ad hoc working group to prepare a risk management evaluation that includes an analysis of possible control measures for hexabromocyclododecane in accordance with Annex F to the Convention for consideration at its next meeting.

This risk management evaluation has now been developed using Annex F information submitted by 16 parties and country observers, including the industry using and producing HBCD. Five non-governmental observers also submitted information. All submissions are available on the Convention website.

The report of the POPRC6 meeting is available on the POPs website:

http://chm.pops.int/Convention/POPsReviewCommittee/POPRCMeetings/POPRC6/tabid/713/mctl/ViewDetails/EventModID/871/EventID/86/xmid/2887/language/en-US/Default.aspx.

2.2.3 World Wildlife Fund

In a survey conducted in 2005 by the World Wildlife Fund (WWF) in different European countries, over 70 persistent, bioaccumulative and/or endocrine disrupting man-made chemicals, including HBCD, were found in the blood of the general population. Based on the findings, the WWF recommended that HBCD be one of the chemicals to be added to the Stockholm Convention, as it fulfills the PBT criteria (WWF, 2005).

2.2.4 United Nations Economic Commission of Europe

In December 2009 HBCD was considered by the Executive Body of the United Nations Economic Commission of Europe (UNECE) Long-range Transboundary Air Pollution (LRTAP) to meet the criteria for POPs, set out in Executive Body decision 1998/2. Based on this decision, the Executive Body has instructed a Task Force on Persistent Organic Pollutants to begin work on the risk management measures, known as the Track B review process. The possible management options for HBCD will provide a basis for later negotiations. The UNECE group includes the European countries, the European Commission, Russia, Canada and the U.S.

The 8th meeting of the UNECE LRTAP Task Force on Persistent Organic Pollutants in Montreal (18–20 May 2010) concluded that the appropriate measure under the POP protocol would be to list HBCD in Annex I to eliminate production and use of HBCD in the UNECE region, with a time-limited exemption for use in EPS and XPS. Because of the potential of releases during recycling of building materials and electronic appliances, parties must take appropriate measures to ensure that recycling processes of articles manufactured or in use by the implementation date to be decided by the Stockholm Convention do not result in recovered material containing 0.1% or more of HBCD by weight in the homogenous material of a product (UNECE, 2010).

2.2.5 OSPAR

HBCD is also covered by the Oslo–Paris Convention for the Protection of the Marine Environment of the North-East Atlantic (the OSPAR Convention). The OSPAR Commission is made up of representatives of the governments of 17 Contracting Parties and the European Commission. An OSPAR Commission background document on brominated flame retardants including HBCD was reviewed by Sweden. OSPAR is committed to base any future decision on HBCD on the conclusions of the EU risk assessment.

2.2.6 Organisation for Economic Cooperation and Development

HBCD was sponsored under the Organisation for Economic Cooperation and Development's (OECD) High Production Volume (HPV) Program by Sweden. An OECD SIDS Initial Assessment Profile (SIAP) for HBCD was discussed and adopted at the 24th OECD SIDS Initial Assessment Meeting (SIAM 24; OECD 2007). Member countries agreed that HBCD possesses properties indicating a hazard for human health with regard to repeated dose toxicity and possible developmental neurotoxicity and for the environment with regard to acute aquatic toxicity to algae, chronic toxicity to daphnia and a high bioaccumulation potential. It was determined that HBCD does not unequivocally fulfil the P criterion of POP: some studies indicated that it does fulfil the criterion, whereas other studies indicated that it does not. However, HBCD is ubiquitously present in the environment also in remote areas far from point sources. The highest concentrations of HBCD are detected in marine top predators such as porpoise and seals, showing that HBCD biomagnifies. The bioconcentration factor (BCF) of HBCD is 18 100 and thus the bioaccumulation criterion is fulfilled. The concern is increased by the trend of increasing tissue concentrations of HBCD in biota, especially marine mammals where for porpoise in the North Sea the concentrations have increased rapidly in recent years.

It was further determined that a "certain flexibility is required in the application of the criteria, for instance in cases where one criterion is marginally not fulfilled but the others are exceeded considerably. This may include for example substances that do not fulfil the persistence criteria but bioaccumulate significantly and are measured in marine biota distant from anthropogenic sources". Overall, HBCD is therefore concluded to fulfil the PBT criteria.

The conclusions of the report were subsequently endorsed by the OECD Joint Meeting of the Chemicals Committee and Working Party on Chemicals, Pesticides and Biotechnology in August 2007. An OECD Hazard Risk Information Sheet was also published in February 2008 (OECD, 2008).

2.2.7 United States

HBCD is listed on the United States (US) Environmental Protection Agency (EPA) Office of Pollution Prevention and Toxic Substances (OPPTS) Index 1 Master Testing List, which is a consolidated listing of chemicals and existing chemical testing priorities (http://www.epa.gov/oppt/chemtest/pubs/index1.pdf).

As part of the Initial Risk Based Prioritisation of High Volume Chemicals project, the US EPA conducted a risk characterization of HBCD based on its environmental fate, hazard and exposure (US EPA, 2008). The information used by EPA included data submitted under the High Production Volume (HPV) Challenge Program and the 2006 Inventory Update Reporting, and data publicly available through other selected sources. The assessment concluded that HBCD has high acute aquatic toxicity to algae and high chronic toxicity to daphnia and also presents human health concerns based on animal test results indicating potential reproductive, developmental and neurological effects. HBCD was ranked low for persistence and high for its poential to bioaccumulate.

Based on its screening-level review of hazard and exposure information, the US EPA released an Action Plan on HBCD in 2010. Under the Action Plan, the EPA intends to initiate the following actions to manage the risk that may be presented by HBCD:

- Consider initiating rule-making under Toxic Substances Control Act (TSCA) to add HBCD to the Concern List of chemicals which present or may present an unreasonable risk of injury to health or the environment. EPA intends to publish this notice of proposed rule-making by the end of 2011.
- Initiate rule-making under TSCA to designate manufacture or processing of HBCD for use as a flame retardant in consumer textiles as a significant new use. This would require manufacturers and processors to notify EPA before manufacturing or processing HBCD for this use. This Significant New Use Rule also would be proposed to apply to imports of consumer textiles articles containing HBCD.
- Consider initiating rule-making under TSCA to regulate HBCD. A s. 6(a) action could take the form of a comprehensive ban on the manufacturing, processing, distribution in commerce and use of a chemical substance, or a more targeted regulation to address specific activities. The extent of the rule for HBCD would be determined during the rule-making process.
- Initiate rule-making in 2011 to add HBCD to the Toxics Release Inventory. Listing on the Toxics Release Inventory will require manufacturers or importers to provide environmental release information.
- Conduct a Design for the Environment assessment for alternatives to HBCD. The information developed may be used to encourage industry to move away from

HBCD instead of, in addition to or as part of any regulatory action taken under TSCA. The alternatives assessment would build upon existing knowledge and would consider various exposed populations, including sensitive human subpopulations, as well as environmental exposure. The work will begin in 2011, with completion expected in 2013.

2.2.8 Canada

Canada has completed a screening assessment of HBCD as part of its pilot program to assess substances on the Canadian Domestic Substances List (DSL). The Screening Assessment Report, published in November 2011, concludes that HBCD meets the criteria set out in s. 64 of the *Canadian Environmental Protection Act 1999*, as it has or may have an immediate or long-term harmful effect on the environment or its biological diversity. The assessment concluded that HBCD demonstrated toxicity in both aquatic and terrestrial species, with significant adverse effects on survival, reproduction and development reported in algae, daphnids and annelid worms. In addition, the assessment concluded that HBCD meets the criteria for persistence and for bioaccumulation as defined by the *Persistence and Bioaccumulation Regulations* made under the *Canadian Environmental Protection Act 1999*, and is a candidate for virtual elimination (Environment Canada & Health Canada, 2011).

Hexabromocyclododecane was added to Sch. 1 to the Act proposing the implementation of virtual elimination of hexabromocyclododecane under subs. 65(3) of the Act.

2.2.9 Japan

The Ministry of the Environment of Japan measured HBCD in air, water, sediment and aqueous biota in 2003. HBCD is classified as a class 1 monitoring substance by the Chemical Substance Control Law. Class 1 substances are those which are not biodegradable and highly bioaccumulative. This classification places an obligation to report manufactured and imported amounts annually.

The Japanese Government is conducting tests on the impact of 1,2,5,6,9,10-HBCD on bird reproduction following an Environment Ministry Policy Commission conclusion that HBCD is bioaccumulative in humans and animals and potentially hazardous. Tests on Japanese quails showed that HBCD deposited in certain species caused reproductive defects. Japan uses about 1000 metric tons of HBCD a year, of which more than 90% is imported. If the testing shows HBCD is toxic, the government will introduce regulations placing conditions on its use, storage and discharge (Chemical Regulation Reporter, 2011).

2.2.10 Voluntary industry programs

Given the risks identified for the environment, and in order to ensure responsible use of the chemical, HBCD producers and users, particularly in Europe, are implementing voluntary programs to monitor, control and reduce emissions to the environment. According to the industry group Bromine Science and Environmental Forum (BSEF), industry has taken initiatives to control emissions from HBCD production sites and from downstream use. In 2003, a voluntary emission reduction program, called the Voluntary Emissions Control Action Programme (VECAP), was initiated to address high levels of HBCD in the environment. Since then, emission levels from production sites have decreased by 99%. Emissions from production in Europe are reported to be negligible. Manufacturers representing more than 87% of HBCD use in the textile sector in Europe have signed up to VECAP. The program has now been extended to downstream users in the textiles and polystyrene foam industries and is expected to further reduce emissions of HBCD into the environment.

The VECAP methodology allows companies to identify possible sources and implement measures to reduce or avoid them (VECAP, 2010).

The sole HBCD production site in Europe, in the Netherlands, has developed methods to control air, water and solid waste emissions. These measures have reportedly resulted in the reduction in 2006 of total HBCD emissions to less than 0.4 grams per tonne. Since 2006, the bromine industry and polystyrene foams producers adopted another voluntary emissions reduction program, SECURE (Self Enforced Control of Use to Reduce Emissions. A vast majority of EPS/XPS producers, representing more than 95% of HBCD consumption, and members of industry federations Plastics Europe and Exiba have committed to this program.

Under its voluntary emission reduction program, BSEF commissioned the Netherlands Institute for Fisheries Research (RIVO) to conduct environmental monitoring and the German Gesellschaft fur Arbeitsplatz (GfR) Institute to quantify water and emissions at selected user industries, covering all known applications. These independent emission measurements showed that emission from HBCD production as well as usage was very low.

In addition to the above-mentioned programs, industry has started a 10-year environmental monitoring program in Europe to monitor the effectiveness of the emission reductions (http://www.oecd.org/dataoecd/3/6/42073463.pdf).

An environmental risk assessment report on HBCD prepared for the European Brominated Flame Retardant Industry Panel assessed HBCD with regard to PBT criteria. The report concludes that, simply because a substance meets screening level hazard categorization criteria and can be detected in remote environment, it does not constitute sufficient justification for concluding that there is a likelihood of significant adverse effects in remote environments. The final judgment to categorise HBCD as a POP requires a more detailed and thorough risk-based assessment (Arnot et al., 2009).

Table 2.1. International regulatory activities on HBCD

Stockholm Convention	Draft risk profile for HBCD was adopted by POPs Review Committee at its sixth meeting in October 2010 (POPRC6). Risk Management Evaluation document under consideration by POPRC7.
OECD	OECD SIDS Initial Assessment Profile (SIAP) for HBCD was adopted by member countries at the 24th OECD SIDS Initial Assessment Meeting (SIAM 24; OECD 2007). Member countries agreed HBCD can be hazardous to human health and the environment.
ECHA	Based on the health effects and in accordance with Directive 67/548/EEC, the EU classified HBCD as follows: Classification: Repr. 2 – H361 (Suspected of damaging fertility or the unborn child) Lact. – H362 (May cause harm to breastfed children) Specific concentration limits: None Labelling: GHS08, Wng, H361, H362 Placed HBCD on the authorisation list, which provides that PBT substances be subject to authorisation. Substances in Annex XIV cannot be placed on the market or used unless authorisation has been granted for a specific use.
UNECE	The 8th meeting of the UNECE LRTAP Task Force on POPs (May 2010) concluded that the appropriate measure under the POP protocol would be to list HBCD in Annex I to eliminate production and use of HBCD in the UNECE region, with a time-limited exemption for use in EPS and XPS (UNECE, 2010).
OSPAR	OSPAR Commission prepared background document on HBCD. Committed to base its decision on EU risk assessment conclusions.
WWF	Recommended that HBCD be one of the chemicals to be added to the Stockholm Convention, as it fulfills the PBT criteria. (WWF, 2005).
USA	The US EPA released an Action Plan on HBCD. The plan includes adding HBCD to the Chemicals of High Concern list and requiring people to notify US EPA before manufacturing or processing HBCD for use in consumer textiles.
Canada	In Canada, HBCD is considered a candidate for virtual elimination following an assessment that concluded that it is persistent and bioaccumulates in organisms.
Japan	HBCD is classified as a class 1 monitoring substance by the Chemical Substance Control Law. Class 1 substances are those which are not biodegradable and highly bioaccumulative. This classification places an obligation to report manufactured and imported amounts annually.

2.3 Australian perspective

HBCD is not manufactured in Australia. Currently there are no restrictions on the manufacture, import and use of this chemical in Australia. HBCD is imported either in a raw form to be formulated for use locally or in the form of articles.

Australia conducted a preliminary PEC assessment of HBCD along with other PBFRs in 2001 (NICNAS, 2001). The report focused on use patterns and potential exposure to PBFRs in Australia. Recommendations included a full assessment of HBCD, focusing on risk to workers handling HBCD, the general public and the environment, and for industry to carefully consider the selection of PBFR compounds for use and ensure that those known to be hazardous are avoided. It was further recommended that labels, safety data sheets and other hazard communication materials be revised to reflect the information on hazards already available for these chemicals (NICNAS, 2001). A survey conducted by NICNAS in October 2004 on the extent of use of brominated flame retardants indicated a significant increase in the use of HBCD in Australia compared to the period 1998–99.

HBCD is currently not listed in Safe Work Australia's Hazardous Substances Information System (HSIS), the Standard for Uniform Scheduling of Medicines and Poisons (Australian Government, 2010) or on the Australian National Pollutant Inventory. There are no environmental restrictions on the use of HBCD in any State or Territory in Australia.

3. Identity, properties and analysis

3.1 Chemical identity

Chemical Name**:** cyclododecane, hexabromo

*CAS Nos 25637-99-4

3194-55-6

EINECS No. 247-148-4

Synonyms: 1,2,5,6,9,10-hexabromocyclododecane

hexabromocyclododecane

Molecular Formula: C_{12}-H_{18}-Br_6

Molecular Weight 641.70

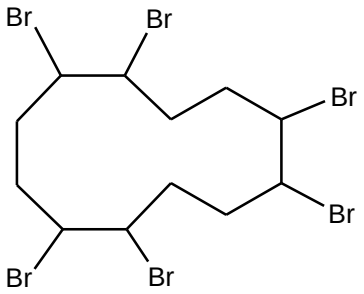
Common Trade Names for Hexabromocyclododecane:

BRE 5300	Pyroguard F 800
Bromkal 73-6CD	Pyroguard SR 103
CD 75	Pyroguard SR 103A
CD 75P	Pyrovatex 3887
FR 1206	Safron 5261
FR 1206HT	Saytex HBCD
HBCD-LM	Saytex HBCD-LM
HBCD-LMS	Saytex HBCD-SF
Myflam 11645	Saytex HP 900
Nicca Fi-None CG 1	SR 103
Nicca Fi-None TS 1	SR 104
Nicca Fi-None TS 3	YM 88

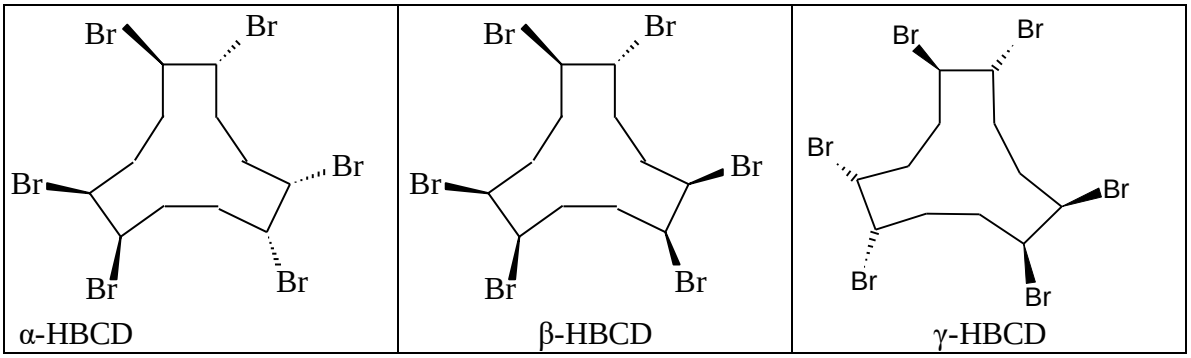
Note: This is not an exhaustive list of trade names.

*The Chemical Abstracts Service (CAS) Registry contains 2 CAS numbers for HBCD: 25637-99-4 for HBCD without any numbering for the position of the bromine substituents; and 3194-55-6 for 1,2,5,6,9,10-HBCD. There are no differences in molecular structure or properties between the chemicals represented by the 2 CAS numbers and both are undefined mixtures of the 3 diastereoisomers, discussed below (Becher, 2004). Both CAS numbers are on the Australian Inventory of Chemical Substances (AICS).

Structural Formula:



Technical-grade HBCD mixtures are obtained via bromination of cyclododeca-1,5,9-triene (CDT) isomers. Considering the 6 stereogenic centres of HBCD, 16 stereoisomers, consisting of 6 diastereomeric pairs of enantiomers and 4 meso forms, can be deduced. Depending on the purity of the starting material and the conditions of the industrial process, a range of technical products with various isomer compositions and melting points are formed. To date, only 3 HBCD diastereomers, α-, β- and γ-HBCD, have been characterised in technical mixtures and the structural formulae for these are shown below (Law et al., 2005). Each of these diastereomers consists of an enantiomeric pair.



Separate CAS Nos. have been reported for α-, β- and γ-HBCD and are as follows (Janak et al.*[2], 2005):

α-Hexabromocyclododecane	134237-50-6
β-Hexabromocyclododecane	134237-51-7
γ-Hexabromocyclododecane	134237-52-8

Spectroscopic and chromatographic data are available for eight of the 16 stereoisomers. Two of the eight isomers showed no optical rotation and are assigned as meso forms (δ and ε HBCD). The 6 stereoisomers identified as 3 pairs of enantiomers are alpha (α) beta (β) and gamma (γ) HBCD (Heeb et al., 2005). In addition, some minor impurities are formed, such as various isomers of tetrabromocyclododecene (Barontini et al., 2001).

[2] References marked with an asterisk were not examined but were quoted as secondary citrations from the key reviews listed in Section 1.3.

The predominant isomer in the commercial product is the γ diastereomer. Based on studies conducted, the commercial product consists of 80%-85% γ, 8%-9% α and 6% β. (ACCBFRIP*, 2005).

Impurities in HBCD are present at less than 4% w/w (EU RAR, 2008). The major impurity in the commercial product is tetrabromocyclododecene. Other impurities observed in tests conducted by the American Chemistry Council's Brominated Flame Retardant Industry Panel (ACCBFRIP) to investigate the potential effects of HBCD were isobutanol and other unidentified compounds (ACCBFRIP*, 2005).

3.2 Physical properties

The physical properties of HBCD are given in Table 3.1.

Table 3.1. Physical properties of HBCD

Property	Value	Reference
Physical state	White, odourless solid	EU RAR, 2008
Particle size	Fine-grade powder (mean size 2-19 μm), standard-grade powder (mean size 20-150 μm), and granular grade (mean size 560-2400 μm).	Information supplied by the applicants
Specific gravity	2.38 g/cm^3	Albemarle Corporation, 2000
	2.24 g/m^3	Great Lakes Chemical Corporation,* 1994
Boiling point	Decomposes at >190 °C	Peled* et al., 2005
Melting point	180–185ºC	Albemarle Corporation, 2000
Vapour pressure	6.27 x 10^{-5} Pa @21ºC	Stenzel* & Nixon, 1997
Water solubility	3.4 (μg/L) @ 25ºC (see comments below)	Stenzel* & Markley, 1997
	48.8 (μg/L) (α-HBCD)	MacGregor* & Nixon, 2004
	14.7 (μg/L) (β-HBCD)	MacGregor* & Nixon, 2004
	2.08 (μg/L) (γ-HBCD)	MacGregor* & Nixon, 2004
Partition coefficient (LogKow)	5.625	MacGregor* & Nixon, 2004
Henry's Law Constant	1.167 x 10^{-4} atm m^3/mol	Calculated (VP/Wsol)
Dissociation constant (pKa)	Not expected to dissociate under normal environmental conditions.	

3.2.1 Water solubility

Stenzel and Markley (1997) determined the solubility of HBCD in water at 25 °C using a column elution method. The test followed OECD TG 105 and EPA 40 CFR § 796.1860 and was conducted according to good laboratory practice (GLP). The test substance consisted of a composite of 3 commercial samples. Analysis of the composite sample showed 93.6% total HBCD consisting of 8.5%, 6.0% and 79.1% α-, β-, and γ-HBCD respectively.

Chemical analysis was performed using high-performance liquid chromatography (HPLC). The limit of quantitation (LOQ) was considered to be 1 ppb (1 µg/L) with a limit of detection (LOD) of 0.5 ppb.

The solubility limit was considered to have been achieved when at least 5 consecutive samples gave a similar result. These 5 samples (at the 2 mL/min flow rate) had a mean concentration of HBCD of 3.4 µg/L. The results from analyses of samples eluted at 1 mL/min had a mean measured concentration of HBCD of 3.3 µg/L. Based on the results from samples collected at both flow rates from the generator column, the solubility of HBCD in water was determined to be 3.4 µg/L at 25 °C, i.e. low water solubility.

A later study (MacGregor & Nixon, 2004) determined the water solubilities of α-, β-, and γ-HBCD using a generator column method following OECD TG 105 and EPA 40 CFR § 796.1860 and conducted according to GLP. The test substance consisted of a composite of 3 commercial samples. Analysis of the composite sample showed 95.9% total HBCD. Separate analytical standards of the diastereomers were obtained as reference compounds.

Concentrations of the separate diastereoisomers in the solute samples were determined by HPLC with mass spectroscopy detection. The LOQ was set at 0.5 µg/L for each diastereomer. The results are shown in Table 3.2.

Table 3.2. Concentration of separate distereoisomers in the solute samples

	Mean aqueous saturation concentrations (µg/L)		
Flow rate	**alpha**	**beta**	**gamma**
1 mL/min	49.9	15	2.21
0.5 mL/min	47.4	14.5	1.96
Overall mean water solubility (ppb)	48.8	14.7	2.08

Desjardins et al. (2004) determined the water solubility of the separate diastereomers in salt water, also using a generator column method to generate salt-water medium. With concentrations measured at the start and end of the study (0 and 72 h), and flow rate and sampling time being 1 ml/min and 30 min, respectively, the solubility of α-, β- and γ-HBCD diastereomers was determined to be 34.3, 10.2 and 1.76 µg/l, respectively. This resulted in a nominal water-soluble concentration of HBCD in salt-water medium of 46.3 µg/l. This water solubility value is used in this assessment when determining systemic exposure to HBCD via dermal route, considering that HBCD dissolved in sweat would be transported across the skin.

These results support the above findings that α-HBCD is in the order of 20 times more soluble than the γ isomer, while β-HBCD is around 6 times more soluble than the γ isomer. Therefore, while γ-HBCD dominates the commercial mixture, when released to water, the total HBCD found in the water column is likely to be dominated by α-HBCD, with rough estimates being 74%, 22% and 4% α-, β- and γ-HBCD in water respectively.

3.2.2 Vapour pressure

The vapour pressure of HBCD at ambient temperature was determined using a spinning rotor gauge (Stenzel & Nixon, 1997). The study was performed according to GLP and followed OECD TG 104 and EPA OPPTS 830.7952. The test substance consisted of a composite of 3 commercial samples. Analysis of the composite sample showed 93.6% total HBCD consisting of 8.5%, 6.0% and 79.1% α-, β-, and γ-HBCD respectively.

The mean vapour pressure of the HBCD sample was determined to be 6.27×10^{-5} Pa with a standard deviation of 0.21×10^{-5} Pa at 21 °C. Based on the scale of Mensink et al. (1995), HBCD is classified as very slightly volatile.

3.2.3 Octanol/water partition coefficient

MacGregor and Nixon (1997) determined the n-octanol/water partition coefficient of HBCD at 25 °C using a column elution method. The test followed EPA OPPTS 830.7560 and was conducted according to GLP. The test substance consisted of a composite of 3 commercial samples. Analysis of the composite sample showed 93.6% total HBCD consisting of 8.5%, 6.0% and 79.1% α-, β-, and γ-HBCD respectively.

Three subsamples of a stock solution containing 0.2% test substance by weight were diluted in acetonitrile/water and analysed to determine the concentration of test substance in octanol. The mean concentration of HBCD measured in the 3 aqueous samples was 3.97 µg/L. The mean concentration of HBCD measured in the 3 octanol samples was 1.67 g/L. Therefore, the K_{OW} was calculated to be 4.22×10^5, or LogKow = 5.625.

Hayward et al. (2006) undertook a comparative evaluation of 3 HPLC based K_{OW} estimation methods for highly hydrophobic organic compounds and included HBCD in the test set of chemicals for 2 of the methods. One of these methods was based on a gradient elution. Elutions were performed at 25 °C on a short Supelco ODP-50 cartridge column (20 mm length) with a flow of 1.8 mL/min. The second method was based on isocratic elutions at multiple temperatures between 5 °C and 45 °C on an ODS-2 column (100 mm length). The elutions were performed isocratically at 80% methanol using 1,3,5-chlorobenzene as a reference standard.

Based on this work, the authors recommend that the first approach is better for estimating the K_{OW} of highly hydrophobic compounds due to its simplicity, speed, precision and accuracy. The first method resulted in isomer specific LogKow values for HBCD being 5.47, 5.07 and 5.12 for γ-, α- and β-HBCD respectively.

The result for γ-HBCD is similar to that determined by Macgregor and Nixon (1997), based on a composite sample consisting mainly (~80%) of the γ isomer. The generator column method is considered particularly useful for measuring K_{OW} for substances with values over 4.5 (United Nations, 2009). Hayward et al. (2006) have noted that there are a number of challenges in using retention behaviour of highly hydrophobic compounds in reverse phase columns. For example, HPLC-based estimation methods rely on the availability of literature values for the LogKow of calibration compounds, which should

be as structurally similar to the target compound as possible. Reliable values for highly hydrophobic chemicals are very rare. United Nations (2009) state that the HPLC method is applicable when the LogKow falls within the range of 0 to 6, indicating it should be acceptable for HBCD.

For the risk assessment, results from both tests may be used. The benefit of the results provided by Hayward et al. (2006) is that separate LogKow values are provided for the α, β and γ isomers and, where considered separately, these values will be used.

3.3 Chemical properties

HBCD is susceptible to thermal degradation. The influence of aluminium on the thermal stability of HBCD was investigated by Barontini et al. (2003). HBCD pyrolysis was carried out using thermogravimetric analysers and a laboratory scale reactor. The results obtained confirmed that the presence of aluminium caused a lower thermal stability of HBCD. However, the presence of aluminium also resulted in a significant increase of the char yield and caused shift towards an exothermic decomposition process. The analysis of the decomposition products showed that the presence of aluminium triggers polycondensation reactions during HBCD thermal degradation.

The technical mixtures of HBCD which consist of 75% to 89% γ-HBCD, between 10% and 13% 20.180det43e and <0.5% to 12% β-HBCD when heated to temperatures above 160 °C demonstrate an interconversion leading to an isomer distribution of 78% α-, 3% β-, and 9% γ-HBCD. Furthermore, it has been shown that HBCD degrades at temperatures above 240 °C (Peled* et al., 1995).

Recently, Heeb et al. (2010) reported changes in HBCD stereoisomer pattern when polystyrenes containing HBCD were subjected to heat. Analysis of the 2 types of HBCD-containing polystyrenes, the low-density EPS and the high-density XPS, revealed that the ratios of the 3 HBCD isomers in the 2 polystyrenes were different. The EPS contained an α:β:γ ratio of 6:13:80 whereas the XPS contained an α:β:γ ratio of 43:36:21.

The HBCD isomer pattern in the EPS changed substantially when the polystyrene was subjected to thermal treatment (prolonged exposure at temperatures of 140 to 160 °C). Proportions of γ-HBCD decreased over time from 80% to 21% in exposed EPS, and those of α-HBCD and β-HBCD increased from 6% to 46% and 13% to 30%, respectively. Whereas the HBCD pattern of the EPS sample changed substantially over time, that of XPS sample remained constant. The XPS sample was already rich in α-HBCDs before thermal exposure and proportions only slightly increased from 43% to 54%, whereas those of β-HBCD and γ-HBCD decreased from 36% to 25% and 21% to 19%, respectively. Thus, the HBCD pattern of EPS and XPS differ considerably before exposure but become similar upon thermal treatment, resembling more and more the pattern of equilibrated HBCD mixtures. This discrepancy in the 2 polystyrenes results from the fact that the XPS is already exposed to higher temperatures than the EPS during production and processing.

Patterns rich in γ-HBCD are typically found in technical HBCD mixtures and those rich in α-HBCD are observed in many environmental samples. This study indicates that thermal exposure of flame-proofed polystyrenes at temperatures of 140 °C to 160 °C induces isomerisation of γ-HBCD to α-HBCD.

3.4 Methods of detection and analysis

In theory, HBCD consists of 16 stereoisomers, 4 meso forms and 6 pairs of enantiomers. Separation of different HBCD stereoisomers is not possible by gas chromatography (GC). HBCD diastereomers interconvert at temperatures >160 ^{o}C. Partial breakdown and even complete loss of HBCD has been reported in GC systems (Law et al., 2005). A relatively broad, unresolved peak is obtained due to thermal rearrangement of the stereoisomers, and results reflect total HBCD concentrations. In contrast to GC, HBCD stereoisomers can be easily separated using reversed-phase liquid chromatography and determined by mass spectrometry (LC/MS or LC/MS-MS). Furthermore, several enantiomers can be resolved on a chiral, permethylated β-cyclodextrin stationary phase for LC. As a consequence, up to eight individual HBCD stereoisomers can now be demonstrated by LC/MS (Covaci et al., 2006).

The decomposition products of HBCD were investigated by gas chromatography / mass spectrometry (GC/MS). HBCD thermal degradation was conducted under a moderate heating rate (10 °C) in a batch reactor using both inert and oxidizing atmospheres. It is known that HBCD decomposition takes place between 240 and 270 °C if the heating rate of 10 °C is used. GC/MS analysis allowed the identification of decomposition products derived from the primary pyrolysis process at the moderate heating rates used. The use of standard samples and the analysis of fragmentation patterns allowed the identification of many products, such as pentabromocyclododecane and tetrabromocyclododecane. Based on the identified products, the main pathways of HBCD thermal degradation were assessed and a mechanism for HBCD decomposition proposed (Barontini et al., 2001).

4. Manufacture, importation and use

The production of HBCD is a batch-process. Elemental bromine is added to cyclododecatriene at 20 to 70 °C in the presence of a solvent in a closed system. The HBCD is used to manufacture flame-retardant products either as a pure substance or as a masterbatch – a concentrated mixture of HBCD encapsulated into a carrier resin such as polystyrene. The manufacture of EPS, XPS and HIPS involves polymerisation, expansion and extrusion processes where HBCD is added in the extrusion process as one of a number of additives. Back-coating to textile is applied by adding a dispersion containing a polymer and HBCD among other additives as a thin coating film.

HBCD is not manufactured in Australia. It was imported into Australia as the raw or technical grade powder or granules, in EPS resin, as liquid dispersions and incorporated into the plastic of finished articles. However, information provided by the applicants in mid 2011 indicate that, since 2010 HBCD is imported into Australia only as liquid dispersions, in EPS resin and incorporated into plastic of finished articles.

4.1 Technical grade HBCD

Prior to 2010, technical grade HBCD was imported mostly as the powder form, under the trade names Saytex HP900P and Great Lakes CD 75P (typical purity ≥96% – 99.9% w/w) and also as a granulated form (99.5% purity w/w) under the trade name FR-1206. HBCD packed in 25 kg polylined paper bags was transported by sea and then as road freight from wharves to importers' warehouses in 6-metre shipping containers and usually packed on pallets wrapped with shrink-wrap plastic. The bags were unpacked and stored in the warehouses on pallets. Those not already wrapped in shrink-wrapped plastic were usually wrapped at this point. The pallets were delivered to customers by road freight.

4.2 Liquid dispersions containing HBCD

Two types of liquid dispersions containing HBCD are imported into Australia for use in textile coating. One of the mixtures is an aqueous dispersion of 15% to 30% HBCD with antimony trioxide. It is imported in high-density polypropylene 200 kg drums, 4 drums to a pallet, in containers, and dispatched to customers by road in trucks with secure caging. The other liquid dispersion is an aqueous mixture containing 30% to 60% HBCD with an anionic surfactant. The mixture is imported in 20 kg steel drums, trucked from the shipping port to the importer's warehouse and is delivered to customers from there.

4.3 Expandable polystyrene resin containing HBCD

EPS resin, in the form of unexpanded beads containing HBCD in concentrations of <1%, is imported in 25 kg polylined paper bags and 700 kg lined meshed plastic bags. The beads are delivered to importers on pallets by road transport and then undergo further processing or are resold.

4.4 Articles containing HBCD

HCBD is also imported into Australia incorporated in extruded polystyrene (XPS) foam boards or in the plastic of finished articles such as printers and projectors.

The boards are imported in bulk shipping containers, shrink-wrapped in plastic and transported by road to building sites. The polystyrene foam boards are used in thermal insulation of houses, commercial and industrial buildings, in the insulation of inverted roofs, and as a core material for structural building panels. HBCD forms <3% by weight of the boards, whose dimensions are approximately 600 mm by 300 mm by 2400 mm. The boards are fixed in place by contractors using mechanical fasteners or adhesives. Some boards may be hand-cut to size and shape on-site and a limited amount of hot wire cutting may be employed by fabricators to produce different thicknesses and shapes. The importing company recommends that the boards be covered by suitable protective building materials – for example, interior or exterior cladding – or located under concrete slabs. Insulated panels are typically clad with steel or aluminium sheet, adhesively bonded to the foam. The service life of the boards is comparable with the life of the building, and a small percentage (<5%) is recovered for reuse at the end of a building's life (estimated at 20 years).

A minimal amount of HBCD was reported to be imported in inkjet printers, projectors, scanners and ventilation units for offices. HCBD has also been reported to be present in some compact fluorescent lamps, at levels of <5 ppm (0.0005%), and LCD digital audiovisual systems, at levels of <1000 ppm (0.1%). There may be other articles containing HBCD imported into Australia that have not been reported – for example, HBCD-treated fabrics and blinds, electric and electronic equipment and also packaging material for various products.

4.5 Quantities imported

Table 4.1 includes import data of HBCD for the years 2003–10. In addition, the data collected earlier, in 1998–99, is also included to demonstrate the trend in the use of HBCD in Australia. The data shows a decrease in the amount of HBCD imported into Australia since 2005–06, when industry had reported imports equivalent to nearly 91 tonnes.

Table 4.1. Quantity of HBCD imported into Australia

Product type	Concentration of HBCD (%)	Total quantity of HBCD (tonnes)							
		1998–99	**2003–04**	**2004–05**	**2005–06**	**2006–07**	**2007–08**	**2008–09**	**2009–10**
Technical grade	~99%	n.d.	12	41.5	51.7	32.0	43	30	0
Liquid dispersions	30%–60%	n.d.	<5	<5	<2	<1	2.0	0.38	0.69
Expandable polystyrene (EPS) resin and beads containing HBCD	0.5%–1%	n.d.	14.5	14.7	30.0	30.0	36	17.0	45.0
Finished articles		n.d.	5.3	6.1	6.4	2.0	5.0	7.7	9.9
Extruded polystyrene	<3%								
Inkjet printer, scanners, printers	<5 ppm	n.d.	n.d.	<1	<1	n.d.	n.d.	n.d.	n.d.
LCD digital audiovisual system	<1000 ppm	n.d.			n.d.	n.d.	n.d.	n.d.	n.d.
TOTAL	**-**	**36.0**	**37.0**	**68.3**	**91.1**	**65.0**	**86.0**	**55.0**	**60.5**

n.d.=no data

The data for HBCD incorporated in articles is not comprehensive, as it is possible that other articles, such as electrical and electronic equipment housed with HBCD-treated HIPS and polymer coated flame-retarded textiles, may be imported into Australia. In addition, HBCD may be entering Australia in packaging material for various products and the quantities of these items are unknown.

4.6 Uses of HBCD

Flame retardants are generally solids and function by intercepting active species, such as oxy-radicals, or by breaking down to release agents which can function in the same way. Any one flame retardant may function by more than one mechanism and useful cooperation effects ("synergism") are obtained with combinations of flame retardants.

HBCD is used as a flame retardant in the moulding of insulation panels, sheets and blocks for use in the construction of industrial and residential buildings. It is also used in plastic products and in polymer dispersions used for flame-retarding textile products. It is an additive flame retardant, which means that it is incorporated into the polymer matrix but does not chemically bond to it. Its properties make it particularly suitable for use as a flame retardant in polystyrene thermoplastic resin, where it can achieve the required flame retardancy at relatively low concentrations compared to other flame retardants. Depending on the application, it can be used in combination with antimony trioxide, which provides a synergistic flame retardant effect.

4.6.1 Use of HBCD in expandable polystyrene resin

The majority of the HBCD imported up until 2010 was used in the manufacture of EPS.

Manufacture of expandable polystyrene resin

The only site manufacturing styrene and EPS using the imported HBCD in Australia, stopped this operation in 2010. The manufacture of EPS involved a suspension process by addition of a blowing agent, usually pentane, which causes resin to foam during moulding, to polyester resin. The process generally occurs in 2 phases.

In the first phase, EPS resin is produced by suspension polymerisation of styrene monomer in water, and impregnation of the polymerised styrene with a blowing agent. This occurs in a batch process involving a single reactor vessel. Styrene monomer and water is charged to the reactor vessel equipped with an agitator, and various chemicals are added to effect suspension of the monomer in water and to control the polymerised bead growth, molecular weight and other parameters necessary to produce the desired product. HBCD and some premixed additives are then added to the reactor either in the granular or powder form. The polymerisation is an exothermic reaction and is controlled by reactor temperatures and pressures as well as various catalysts.

In the second phase of the process, the blowing agent is added under pressure and impregnates the soft polystyrene beads. The resultant beads are then subjected to steam heating to above their glass transition temperature, resulting in the beads expanding (by 40 to 80 times) and producing the cellular form.When completed, the entire batch, consisting of water and the impregnated polystyrene beads, is dumped to de-watering systems; the beads are then transferred to dryers. The dried beads are screened to provide the desired size cut. Various surface lubricant systems are then added to aid in screening and to eliminate potential lumping during pre-expansion.

Pre-expansion and conditioning process

The purpose of pre-expansion is to produce foam particles (prepuff) of the desired density for a specific application. During pre-expansion, the EPS beads are fed to a vessel equipped with a controlled steam inlet, an air inlet and an agitator (this vessel is called a pre-expander).

By controlling the bead feed rate, steam, air flow and agitator speed, the quality of the prepuffs can be controlled. Softening of the bead occurs at around 90 ºC , which is above the normal boiling temperature of the blowing agent (pentane). The internal vapor pressure causes the beads to expand to the required density. This initial pre-expansion process can be carried out in either a continuous or a batch operation. In the pre-expander process, EPS beads are introduced continuously by way of an endless screw at the bottom of the pre-expander, and the expanded beads (prepuff) come out at the top (similar to a popcorn popper).

In the batch process, the beads are loaded from the top and emptied after the operation at the bottom of the apparatus. Both the continuous and the batch processes are used in Australia. The prepuff exiting the pre-expander is pumped into a holding silo to mature. The prepuff is normally emptied from the prefoamer into a fluid bed dryer before it is transported in a stream of air (airveyed) to the holding silo. The fluid bed dryer normally removes almost all of the surface moisture from the prepuff. From the silo the EPS prepuff is either shape moulded or block moulded.

The majority of HBCD flame-retarded EPS resin, both manufactured in Australia and imported as unexpanded EPS beads, is used in the moulding of insulation panels, sheets and blocks for use in the construction of industrial and residential buildings. In block moulding, moulds made of stainless metal are closed and the prepuff is transported in a stream of air into the mould cavity. A vacuum pump lowers the pressure, resulting in elimination of air and water, and the mould is then subjected to steam pressure, causing fusion of the prepuff. The block is cooled and ejected. The temperature of the block when ejected is about 90 to 95 ºC. Shape moulding is conducted using similar principles to block moulding. Shape moulding allows for EPS to be moulded simultaneously with another plastic film, if required.

End use of EPS materials containing HBCD

Insulation panels

Shaped blocks are used in buildings as insulation panelling in various ways, including as exterior sheathing, exterior walls, ceiling, upside-down roofs (protected membrane roofs) and subfloor systems. Moulded EPS blocks can be shaped at the building site, most often with a bandsaw, or they can be shaped in factories using hot wire cutting techniques. They can be combined with other materials such as steel (as in sandwich panels – factory-engineered units typically comprising 2 metal faces bonded to a fully insulating core, commonly used for coldstore construction, in both freestanding and load-bearing panels), concrete (as an insulation core in tilt-up wall panels) and gypsum and plasterboard (as skins for ceiling panels and other prefabricated components). It is sometimes faced with reflective foil and used in conjunction with an airspace to enhance the total thermal resistance of the system. Its high resistance to water absorption and mechanical strength makes it suitable for this application (Kirk-Othmer, 1984).

Packaging

EPS resins in Australia are also used for the manufacture of miscellaneous industrial packaging, including packaging used for whitegoods. It is reported that some appliance and computer manufacturers use flame-retarded EPS resin to reduce fire hazard in warehouses.

Beanbag filling

Some imported EPS resin is processed in Australia to make foam for beanbag filling.

Other uses

Other applications of HBCD flame-retarded EPS resins in Australia include automotive uses – for example, as insulation in vans, as filler in bumper bars, in the manufacture of baby seats and in advertising signage.

4.6.2 Use of HBCD in the textile industry

Imported liquid dispersions of HBCD are used to formulate polymer coatings for textile coating in Australia. No formulation of polymer dispersions from powdered HBCD for textile coating was identified in Australia.

Blinds

Imported liquid dispersions of HBCD are used in polymer coatings applied to polyester and polyester/cotton blend fabrics used to manufacture vertical, holland and roman blinds used for window shading in domestic residences and other buildings. The formulation containing 2% HBCD is carried out at one site in New South Wales in Australia. The formulated coating dispersion is pumped from a drum and applied to the polyester fabric using a doctor blade. The fabric then moves through a series of ovens (up to 150 °C) to dry the coated materials. This process is repeated to apply several coats of the fire-retardant formulation to be applied to each side of the fabric. Approximately 9 to 13 tonnes of flame-retardant liquid dispersion product is used annually for this purpose.

Automotive and technical textiles

HBCD is formulated to polymer-based dispersions (e.g. acrylic or latex) of variable viscosity in the polymer industry. The dispersions are then processed in the textile finishing industry. Application is generally between 10% and 20% of the liquid dispersion product on weight of fabric. Exhaustion of the dispersion onto the fabric is believed by the manufacturers to be 95% to 99%. Fabric treated is 100% polyester. These textiles are used in automotive seating products, public seating products and protective garments for military and industrial purposes. Approximately 2 to 3 tonnes of flame-retarded liquid dispersion product are used annually for this purpose.

4.6.3 Use of HBCD in other plastics

A small amount (<5%) of imported raw HBCD powder was used in the manufacture of a flame-retarded polystyrene masterbatch. HBCD was added to the blend at a concentration of 50% and the masterbatch is extruded into granules 2 to 3 mm in diameter. The masterbatch is used in the manufacture of ceiling fan covers in an injection moulding process.

A minor application of HBCD is in HIPS, which is used in electrical and electronic equipment and appliances (e.g. audiovisual equipment).

4.6.4 Use of HBCD in articles

Imported finished articles which contain HBCD in plastic parts include XPS insulation boards, office equipment such as inkjet printers, projectors, scanners and ventilation units for offices. Other manufactured or semi-manufactured articles containing HBCD flame-retarded plastics may be imported into Australia.

4.6.5 Summary of uses in Australia

The distribution of HBCD by use pattern based on the imported quantities is provided in Table 4.2. Unreported amounts may include HBCD in imported HBCD-treated textiles and articles made from EPS or other plastics containing HBCD, as in packaging.

Table 4.2. Distribution of HBCD by use pattern

Form	Use	% use of total imported HBCD
Expandable and extruded polystyrene resin (EPS)	Domestic and industrial building insulation; Packaging for industrial products; beanbag fill; other (incl. automotive)	>88%
Other resins	Housing for electrical appliances	<5%
Textile coating additive	Blinds, public seating, garments	5%
Unspecified plastics in imported finished articles	Inkjet printers, projectors, scanners, ventilation units	<1%

Information provided for the application of imported EPS resin indicated that the majority is used in house insulation, exterior walls and sandwich panels (see Table 4.3). No information was provided on the proportionate breakdown of applications of EPS resin manufactured in Australia.

Table 4.3. Applications of EPS resin as percentage of total imported EPS resin

Application	% of imported EPS resin
House insulation, exterior walls, cool store sandwich panel	75%
Packaging for miscellaneous industrial products	15%
Beanbag fill	10%

4.6.6 Other uses overseas

Other potential uses of HBCD, not reported to occur in Australia but identified by manufacturers of HBCD and in overseas reports, include as a flame retardant in foamed polystyrene crystal, SAN (styrene-acrylonitrile) resins, adhesives and coatings (Albermarle, 2000). HIPS-containing HBCD used for video or audio equipment housings is reported to be a very minor application, as generally HBCD is not used to flame retard electronic housings (e.g. television sets) due to the level of flame retardancy required by manufacturing standards for these products.

Other polymers in which HBCD is reported to have applications are latex binders; unsaturated polyesters; and polyvinyl chloride wire, cable and textile coatings (National Research Council, 2000).

Textile types in which HBCD has been reported to be used include draperies and wall coverings (FRCA,* 1998). It is also reported to be used in coatings and adhesives (Albermarle, 2000). Technical brochures for EPS suggest HBCD use in manufactured products for flotation, geotechnology, product displays and stage settings (Huntsman Technical Bulletin 1- 1.0 'General Introduction to Expandable Polystyrene (2006)).

The EU Risk Assessment Report (EURAR, 2008) provides the following list of end products in Europe that may contain HBCD:

- flat and pile upholstered furniture (residential and commercial furniture)
- upholstery seatings in transportation, draperies, and wall coverings
- bed mattress ticking
- interior textiles e.g. roller blinds
- automobile interior textiles
- car cushions
- insulation boards (against cold or warm) of transport vehicles e.g. lorries and caravans
- insulation boards in building constructions e.g. house walls, cellars and indoor ceilings, and "inverted roofs" (outdoor)
- parking decks
- insulation boards against frost heaves of road and railway embankments
- packaging material (minor use and not in food packaging)
- electrical and electronic equipment e.g. distribution boxes for electrical lines
- video cassette recorder housings
- polyvinyl chloride wire, cable and textile coating
- protecting paints for military purposes.

5. Public exposure

5.1 Exposure assessment methodology

The purpose of this evaluation is to determine the magnitude of public exposure to HBCD as well as the frequency and duration of that exposure. This requires an understanding of the routes by which exposure occurs over the life cycle of HBCD, together with an understanding of the variability of consumer exposure as a result of differing use patterns and environmental conditions.

Public exposure includes direct exposure through use of consumer products and indirect exposure via the environment. Consumer exposure is assessed based on the typical scenarios that a consumer may encounter. Exposure through the environment is assessed based on measured or predicted data of HBCD in the different environmental compartments and in food and drinking water. Exposure via dust released by HBCD-containing articles within a household is considered under indirect exposure.

Public exposure to a chemical is not uniform across a population. Some groups or individuals may have higher potential exposures due to their locations (such as living in the vicinity of industrial sources), their water and food supply, their dietary habits, personal preferences on certain end-use products, and age-specific behaviours such as inadvertent soil ingestion among young children through mouthing of objects or hands.

In this assessment, exposures are estimated separately for adults and children where relevant. All the adults are considered to be one group, and subgroups such as prospective parents or elderly people are not considered separately. Exposures to children are estimated for 3 representative age groups: infants (1–6 months), toddlers (2 years) and children (12 years). The children's age groups are selected across several key life stages (infancy, childhood, and adolescence) and take into account the differences in exposure with the life stages.

Exposure for each age group is estimated by using typical and reasonable worst-case exposure scenarios. It is believed that this approach will address practically all exposure within a population. However, it should be noted that there may still be uncertainties associated with such exposure estimates, although care has been taken to address and document them.

Actual measured data are always preferable in an exposure assessment. Where Australian data were not available, overseas data were used. Modelled data were used only when no measured data were available (Appendix 3).

5.2 Direct exposure

5.2.1 Sources of exposure

HBCD is used in consumer products as an additive flame retardant; that is, it is present physically in the articles rather than chemically bonded. It is possible for HBCD to be released to some extent from the treated articles. Consumers who use these treated products may therefore be directly exposed.

Consumer products/articles containing HBCD are mostly imported. Articles manufactured in Australia that are likely to contain HBCD are listed in Section 4.

The HBCD in these products/articles ranges from less than 5 ppm to 1000 ppm. Overseas data indicate that imported products, such as upholstered furniture (residential and commercial), textiles used for draperies and wall coverings and bed mattress ticking may also contain HBCD.

5.2.2 Estimated direct exposure

Oral

It is unlikely that materials detached from HBCD-treated articles will be ingested. Children may mouth polystyrene packaging and beanbag filling; however, owing to very small concentrations of HBCD in these polymers (<0.7%) and its low solubility in water, direct consumer exposure through ingestion is considered negligible.

Inhalation

Due to its low vapour pressure (6.3×10^{-8} kPa at 21 °C), significant emission of HBCD vapours from treated articles is not expected. Low emissions from treated articles are demonstrated by 2 overseas studies.

Kemmlein et al. (2003) reported an emission study of a range of flame-retarded articles conducted by the German Federal Environmental Agency. In this study, insulation foams made of EPS (1–2% HBCD) and of XPS (<1% HBCD) and HBCD-treated upholstery fabric were placed separately in emission chambers for 105 to 168 d under standard climatic conditions (temperature = 23 °C, relative humidity = 50%). For the insulation foams, a trace amount of HBCD (i.e. 1–3 $\mu g/m^2$) was found adsorbed on the interior surfaces of the chambers after 105 d of testing, but it was not detected in the chamber atmosphere (detection limit: 0.33 ng/m^3). A very low emission rate of 1 to 4 $ng/m^2/h$ was thus calculated for insulation foams made from EPS, and 0.1 to 29 $ng/m^2/h$ for XPS. For the upholstered fabric, no emissions were detected for up to 168 d of testing.

Klatt (2004) passed purified air through a HBCD-treated XPS tube (containing 1.1 to 2.0% HBCD) at a rate of 15 L/h and measured HBCD emissions from the other end. Measurements were made at 5, 10, 15 and 30 d, using LC-MS with a detection limit of 5 ng per sample. HBCD was detected only on the first sampling (on day 5), at about 10 ng per sample. The author suggests that only the outer layer of the treated article could emit HBCD to the surrounding atmosphere; HBCD additive under the surface cannot be emitted because of "restricted mobility". A very low emission rate of 17 $\mu g/m^2$ per year was predicted.

EPS resin in Australia is predominantly used in insulation boards in industrial and residential buildings. These boards are typically used within wall cavities for insulation purposes and are usually covered with materials such as aluminium or steel cladding, concrete, fibreglass meshing, polymer-cement composites, paint, tiles or wallpaper. Given the low vapour pressure of HBCD, its very low emission from treated articles, and its use mainly in enclosed and non-accessible locations, consumer exposure through inhalation is considered to be negligible.

Dermal

Occasional or infrequent skin contact with some HBCD-treated products (for example, beanbag filling, packaging material, insulation panels, plastic backing for blinds and plastic electronic casings) should result in very low dermal exposure. However, direct and frequent skin contact with treated textile articles (such as automotive textiles, textiles for public seating and child seats, and specialised protective clothing) may result in higher dermal exposure.

Dermal exposure is estimated for this situation which represents a reasonable worst-case exposure scenario for consumer exposure to HBCD.

Based on a survey of South Australian residents, the mean number of hours spent in a car per day on weekdays was 0.92 h, with a median value of 0.60 h; and on weekends 1.07 h with a median value of 1.0 h (enHealth, 2003). The time spent in cars by children below 11 years, as reported in the Hubal study (US EPA, 2002) is 1.1 to 1.6 h per day. For estimation of exposure, time spent in a vehicle is assumed to be 1 h per day for both children and adults.

In estimating dermal exposure from automotive upholstery, it is assumed that 25% of the skin surface area of an adult's thighs and trunk would be in contact with the seat (National Research Council, 2000). The maximum concentration of HBCD in sweat is assumed to be 0.0463 μg/cm^3, based on its solubility in salt water (see Section 3.2), and the dermal absorption is assumed to be 4%. The standard body surface areas for adults and children have been used (US EPA, 2002).

Based on the above assumptions, the systemic exposure from dermal contact with treated automotive upholstery is estimated as follows (for detailed calculations, see Appendix 3, Scenario 1).

Table 5.1. Estimated dermal exposure to HBCD from automotive textiles

	Bodyweight (kg)	**Surface area of exposed skin (cm^2)**	**Time spent in vehicle (h/day)**	**Worst-case estimate (ng/kg bw/d)**
Infants (1–6 months)	5.8	333	1	0.04
Toddlers (2 years)	12.9	606	1	0.04
Children (12 years)	46.9	1594	1	0.03
Adults	60.0	1918	1	0.03

It should be noted that the above estimates represent the worst-case scenario. In practice, dermal exposure is expected to be less, as only a small proportion of the skin would continuously produce sweat. Also, wearing of more clothing in colder climates reduces the area of exposed skin, additionally HBCD is applied to the back of textiles and this further reduces dermal exposure.

5.3 Indirect exposure

5.3.1 Sources and routes of exposure

HBCD can be released in the general environment through release into the atmosphere or wastewater from its industrial uses and disposal, and leaching and emission from landfill. Local environments such as inside houses or cars may contain HBCD released from HBCD-treated articles, and this will also eventually be transported to the general environment.

The distribution of HBCD into the different environmental compartments (air, water, soil and sediment) is described in Section 7. HBCD can migrate from soil and sediment into the aquatic and terrestrial food chains, ultimately ending up at higher concentrations in the lipid portion of food.

Local environments, such as house interiors, may have different levels of HBCD as compared with general environmental compartments. In addition, indoor levels vary due to the variable presence of HBCD-containing articles within different houses.

Indirect exposure of humans to HBCD in the environment may occur by consumption of food and drinking water, inhalation of air and ingestion of soil and dust (particularly by children). Quantitative exposure assessment has been conducted for each of the following scenarios:

- inhalation exposure from indoor air
- inhalation exposure from outdoor air
- oral exposure from the ingestion of soil and indoor dust due to hand-to-mouth behaviour, particularly in children
- oral exposure from the consumption of food and drinking water
- oral exposure caused by the consumption of breast milk by infants.

Indirect exposure through dermal contact – for example, with soil – can occur. However, exposure via this route is considered to be negligible.

5.3.2 Indoor exposure

Inhalation exposure from dust

Due to the low volatility of HBCD, the inhalation exposure from the indoor air environment is considered to be solely from HBCD in dust. Inhalation exposure to HBCD from dust may be estimated if the concentration of HBCD in dust and the amount of dust in the air are known. HBCD has been found in dusts collected in houses and offices in many countries and these studies are summarised in Table 5.2. There are no Australian studies on indoor dust level of HBCD.

The studies typically used a vacuum cleaner to collect dust from the floor of the house or office with HBCD concentrations determined by HPLC or GC/MS. There were differences in particle sizes of the dusts analysed as well as the reporting of the results of each study (e.g. pooled or individual samples).

Table 5.2. Measured levels of HBCD in dust in houses and offices

Location of sampling	N	HBCD concentration (μg/kg dust)			Comment	Reference
		Mean (Median)	90th percentile	Range		
Parliament building in 8 EU countries and internet provider office	16	(560)	1800	<3–3700	Settled dusts were collected with analysis of particles <1000μm; HBCD detected in 13/16)	Santillo et al., 2001; Leonards et al., 2001
100 homes in the UK – settled dusts pooled into 10 regional samples	10	3158	NA	940–6900	Only dust particles <2000 μm were analysed; HBCD was detected in all samples	Santillo et al., 2003a
5 homes in Germany – settled dusts pooled into 1 sample	1	1200	–	–	Only dust particles <2000 μm were analysed. HBCD was detected in all 14 samples	Santillo et al., 2003b
22 homes in Spain – dusts pooled into 4 regional samples	4	225	NA	190–850		
58 homes and schools – dust pooled into 8 samples	8	485	NA	77–1600		
5 homes in Italy – settled dusts pooled into 1 regional sample	1	250	–	–		
17 houses in the US	17	(140)	–	<3–925	Settled dusts were collected. Only particles <1000 μm were analysed	Stapleton et al., 2004
UK homes	31	6000 (730)	NA	140–110 000	One m^2 of carpet was vacuumed for 2 min in each location, in case of bare floors 4 m^2 was vacuumed for 4 min	Abdallah et al., 2008
UK offices	6	1400 (650)	NA	90–3600		
Canadian homes	8	670 (340)	NA	64–1300		
US homes	13	810 (390)	NA	110–4000		
20 US homes (living rooms)	16	354	NA	<4.5–130 200	HBCD levels in bedrooms and home vacuum bags found lower than those in living rooms	Stapleton et al., 2008
43 homes selected randomly throughout Belgium	43	1735 (130)		5–42 692		D'Hollander et al., 2010
10 offices selected randomly throughout Belgium	10	592 (367)		256–1153		
14 homes in Romania (10 in Iasi city and 4 in rural area)	14	(190)		30–600		Dirtu & Covaci, 2010
Classrooms in child day care centres and primary schools in UK	43	8900 (4100)	37 000 (95th percentile)	72–89 000		Harrad et al., 2010

Given the extreme variation of up to 5 orders of magnitude between individual houses, classrooms, and offices seen in Table 5.2, pooled studies give limited information. The studies of individual premises allow the entire distributions of HBCD house dust concentrations to be determined, if sufficient premises are sampled. There are recent data available for between 31 and 43 homes in the UK, US and Belgium, which indicate that the median dust concentration is higher in the UK than in the other countries. The occurrence of extreme values (>40 000 µg/kg) was noted in all 3 Western Hemisphere countries, and dust concentrations of HBCD in this range are therefore expected to also be relevant to Australia.

The HBCD measurements in dust conducted by Abdallah et al. (2008) and Harrad et al. (2010) in UK homes, classrooms in childcare centres and primary schools, and offices were combined, and are presented in Figure 5.1. The individual levels of HBCD in the 80 dust samples exhibit a log normal distribution. In these circumstances, the mean value is strongly affected by variations in the few values at the high concentration end, particularly where a comparatively small number of samples are tested. The median value is much less affected by changes in the small number of higher concentration results. Results must be treated with caution when the reporting involved the use of pooled samples, for which a median value cannot be derived. For a log normal distribution of individual concentrations, results reported as medians are expected to be much lower than those reported as means.

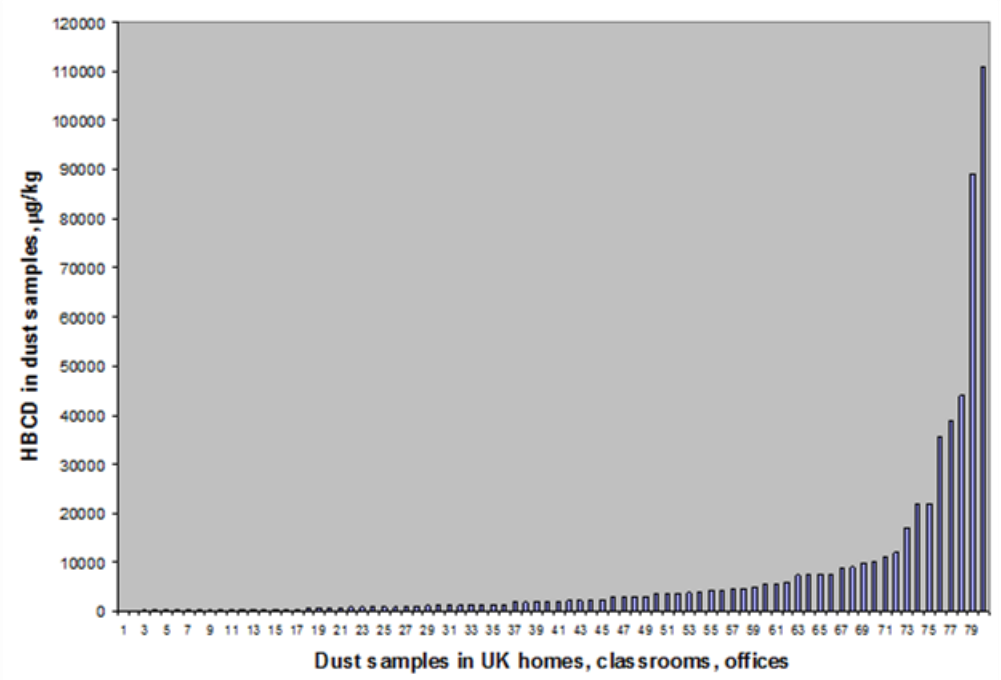


Figure 5.1. HBCD concentrations in dust samples in UK homes, classrooms and offices from Harrad et al. (2010) and Abdallah et al. (2008).

Further analysis of the HBCD concentrations in the UK dust samples gave mean, median, 75th percentile, and 95th percentile values of 7249, 1976, 5450, and 35 630 µg/kg. Although the median could be chosen as the typical exposure value, this would greatly underestimate the exposure in the premises where concentrations are above the median. It is considered that the 75th percentile is more appropriate to assume as the typical scenario to take into account the high variability of the results. The 95th percentile HBCD level from the dust samples analysed from Abdallah et al. (2008) and Harrad et al. (2010) studies is chosen as the reasonable worst-case value.

It should, however, be noted that, for a log normal distribution, the 95th percentile is most likely a significant underestimate of the higher percentiles, which is not the case for normally distributed values, and this will be addressed in the risk characterisation (Section 10.2).

The worst-case estimate of the amount of dust in indoor air is based on the maximum permissible level of particles in indoor air of 90 $\mu g/m^3$, as recommended by Australia's National Health and Medical Research Council (NHMRC, 2004). Considering the HBCD levels in dust from the investigations by Abdallah et al. (2008) and Harrad et al. (2010), the 75th (typical value) and 95th percentile (reasonable worst-case value) HBCD concentrations in dust are equivalent to HBCD concentrations in air of 0.49 and 3.21 ng/m^3 as the typical and reasonable worst-case levels, respectively.

Estimation of inhalation exposure

In estimating inhalation exposure to indoor dust, the following assumptions are made:

- 75% of the inhaled dust will be retained in the respiratory tract and 25% will be exhaled (enHealth, 2002)
- the ventilation rates for infants, toddlers, children and adults are 0.8, 3.8, 15 and 22 m^3/day, respectively (enHealth, 2003)
- indoor air exchange rate is negligible
- the time spent indoors is 20 h/d for both adults and children in Australia (enHealth, 2003)
- the bioavailability for inhalation exposure is 100%.

The size distribution of dust particles containing HBCD is unknown. The Environmental Health Committee publication *Environmental health risk assessment guidelines for assessing human health risks from environmental hazards* (enHealth, 2002) states that half the inspirable dust will be sufficiently small to reach the pulmonary alveoli. It is assumed as a worst-case scenario that all the retained dust particles containing HBCD are available for absorption, either within the lungs or in the gastrointestinal tract following mucociliary clearance.

The exposure arising from the inhalation of HBCD in indoor air is estimated by using the method and equation provided at Appendix 3, Scenario 2, and the results are presented in Table 5.3.

Oral exposure from ingestion of indoor dust

The ingestion of dust/soil is a potential source of human exposure to chemicals. Adults may ingest soil or dust particles that adhere to food, cigarettes or their hands. The potential for exposure via this route is greater for young children because they are more likely to ingest soil than adults as a result of behavioural patterns during childhood. Inadvertent dust ingestion among young children may occur through mouthing of objects or hands.

Deliberate dust or soil ingestion is defined as pica and is considered to be relatively uncommon. As such, pica behaviour will not be considered in this assessment.

The enHealth Council has reviewed the relevant studies on soil ingestion behaviour and recommended the following soil ingestion rates: negligible for infants aged 0 to <1 years, 100 mg/day for children aged 1 to <5 years, 50 mg/day for children aged 5 to 15 years, and 25 mg/day for adults (enHealth, 2003). These values were applied to indoor dust in the estimation of oral exposure.

The soil ingestion rates cited by the enHealth Council as well as the US EPA in their exposure factors handbooks are intended to represent ingestion from a combination of soil and dust, without distinguishing between these 2 sources. The recommended default values are called "soil" which also include "dust tracked into the indoor setting, indoor settled dust, and air suspended particulate matter that is inhaled and swallowed" (enHealth, 2003).

Estimation of oral exposure

In the estimation of oral exposure via ingestion of HBCD in dust, the following assumptions are made:

- The typical and reasonable worst-case HBCD concentrations in dust for the general population are 5.45 and 35.63 μg/g, respectively.
- Dust/soil ingestion factors in Australians are based on the information from enHealth (enHealth, 2003).
- On average, both adults and children spend 20 h/d indoors (enHealth, 2003). The source of indoor exposure is solely from dust.
- Average bodyweights for infants, toddlers, children and adults are 5.8, 12.9, 46.9 and 60 kg, respectively.
- The bioavailability for oral exposure is 100%.

The exposure arising from the ingestion of soil or dust due to hand-to-mouth behaviour can be estimated by using the method and equation provided at Appendix 3, Scenario 3.

Total indoor exposure

The combined typical and reasonable worst-case exposure estimates for the indoor environment for the general public are presented in Table 5.3.

Table 5.3. Estimate of typical and reasonable worst-case indoor exposure from inhalation of dust and ingestion of soil and dust

Age group	Inhalation exposure (ng/kg bw/d)		Oral exposure (ng/kg bw/d)		Total exposure (ng/kg bw/d)	
	Typical	Reasonable worst-case	Typical	Reasonable worst-case	Typical	Reasonable worst-case
Infant (1–6 months)	0.04	0.28	Negligible	Negligible	0.04	0.28
Toddler (2 years)	0.09	0.59	35	230	35	231
Child (12 years)	0.10	0.64	4.8	32	4.9	33
Adults	0.11	0.74	1.9	12	2.0	13

The estimation shows that the ingestion of dust is the major contributor to exposure and therefore the highest level is seen in toddlers (2 years), followed by children (12 years).

5.3.3 Outdoor exposure

Inhalation exposure from dust

No Australian monitoring data are available for HBCD in air. Local predicted environmental concentration $(PEC)_{air}$ values of 62.5–97 pg/m^3 were calculated using worst-case assumptions that all processing occurs in either of Australia's 2 major cities (see Section 7.4.1). For exposure estimate, a reasonable worst-case PEC_{air} of 97 pg/m^3 is used.

Estimation of inhalation exposure

In estimating inhalation exposure of the public to HBCD in outdoor air, the assumptions are the same as that for estimating the inhalation exposure to indoor dust with the additional assumption that the time spent outdoors is 4 h/d (enHealth, 2003).

The exposure arising from the inhalation of HBCD in outdoor air is estimated by using the method and equation provided at Appendix 3, Scenario 2, and is presented in Table 5.4.

Oral exposure from ingestion of soil

No Australian monitoring data are available for HBCD in soil. A one-year PEC_{soil} of 0.37 mg/kg soil was calculated for the case of agricultural soils amended with sewage sludge. The 10-year accumulation level is 3.7 mg/kg (or 3.7 μg/g) soil (Section 7) and is considered as the reasonable worst-case value. It is expected that urban soils would show lower HBCD levels than amended agricultural soil.

Estimation of oral exposure

In estimating oral exposure of the public to HBCD in outdoor soil, the assumptions are the same as that for estimating the oral exposure to HBCD in indoor dust with the additional assumption that the time spent outdoors is 4 h/d (enHealth, 2003).

The exposure arising from the ingestion of soil due to hand-to-mouth behaviour is estimated by using the method and equation provided at Appendix 3, Scenario 3.

Total outdoor exposure

The combined exposure estimates from the outdoor environment are presented in Table 5.4.

Table 5.4. Estimate of reasonable worst-case outdoor exposure from inhalation of dust and ingestion of soil and dust

Age group	Inhalation exposure (ng/kg bw/d)	Oral exposure (ng/kg bw/d)	Total exposure (ng/kg bw/d)
Infant (1–6 months)	0.002	Negligible	0.002
Toddler (2 years)	0.004	4.8	4.8
Child (12 years)	0.004	0.7	0.7
Adults	0.004	0.3	0.3

The estimation shows that the ingestion of soil is the major contributor to exposure and therefore the highest level is seen in toddlers (2 years), followed by children (12 years).

5.3.4 Food consumption

Dietary intake of a chemical is estimated by combining food consumption data with the concentration of the chemical in the food. There are no Australian total HBCD dietary studies available. Total HBCD dietary intakes for the Australian population are estimated from the international studies wherein the dietary exposures to BFRs were estimated based on total diet studies analysed for these compounds.

The UK Food Standards Agency estimated the dietary exposures to BFRs and related compounds in UK consumers (Food Standards Agency, 2006). This study analysed 19 composite samples of food groups collected during the 2003 UK Total Diet Survey for polybrominated diphenyl ethers (PBDEs) and brominated dioxins. However, it was concluded that this was an insufficient sample to obtain results of good quality for HBCD. These compounds were therefore remeasured in samples from the 2004 UK Total Diet Survey. Foods analysed included various fruits and vegetables, dairy products, meats, cereals and oils. Upper bound estimates were presented for "average level consumers" and "high level consumers" of various food groups and a total dietary exposure was derived from a distribution of the individual consumer's consumption patterns across all foods.

The European Food Safety Authority (EFSA) published its Scientific Opinion on Hexabromocyclododecanes (HBCDDs) in Food (EFSA, 2011) which provided an analysis of HBCD in food samples from across 32 dietary surveys compiled from the monitoring programs of European countries in the period 2000-2010. The dietary surveys were entered into a comprehensive database and the HBCD total dietary exposures, reported as minimum lower bound and maximum upper bound for average and high (95^{th} percentile) consumers, were estimated for different age groups. EFSA acknowledged in their methodology that deviations observed in dietary exposure between surveys were influenced by varying food consumption patterns and survey methodologies on the different European countries.

The Dutch National Institute for Public Health and the Environment estimated the dietary exposure to BFRs, PBDEs, HBCD and TBBPA (de Winter-Sorkina et al., 2003). Estimates of dietary exposure were calculated by using concentration data from The Netherlands Institute for Fisheries Research (RIVO) and consumption data from the third Dutch National Food Consumption Survey. The concentrations of HBCD and TBBPA in 91 samples from various food categories were determined. Food products analysed included dairy and dairy products, eggs, meat and poultry, animal fats, fish and vegetable oil. HBCD was present in 15 of the 18 categories of foods. The dietary exposures were based on mean consumption data per food group and mean compound concentration. A high percentage of samples (60% to 91%) were below the LOD, so the estimated exposures to individual BFRs (HBCD and TBBPA) were determined by calculating 2 scenarios. These were based on either a "middle" scenario where samples <LOD = 0.5 x LOD or a "low" scenario where samples <LOD = 0, as very high detection limits were reported for some samples. The high percentage of non-detects strongly influenced the outcome of the dietary exposure estimates in this study and the difference between the results of the 2 dietary exposure scenarios was large for most BFRs.

The levels of HBCD and TBBPA were measured in human milk and total diet surveys from 12 provinces in China in 2007 (Shi et al., 2009). Forty-eight total diet samples were collected and analysed for the diastereoisomers of HBCD and TBBPA and the mean daily intake was then estimated. The types of food consisted of egg and egg products, aquatic

foods, milk and milk products, and meat and meat products. HBCD was detected in approximately 70% of the 662 food samples, with the highest concentration found in the aquatic food products. The medium bound estimate of adult dietary exposure to HBCD was reported as being 0.5 x LOD.

The dietary intakes of the diastereoisomers of HBCD were evaluated in the Belgian population (Goscinny et al., 2011). The 549 food items were categorised into 5 food groups: dairy products, meat and meat products, eggs, fish and fishery products, and other products. The food groups relevant to the study were identified from the Flemish food consumption survey with food items specific to the region. The dietary exposures were estimated from the levels of HBCD detected in the food and the food consumption data from the diet surveys.

A summary of the HBCD dietary exposures is presented in Table 5.5.

Table 5.5. Comparison of estimated dietary exposures to total HBCD from various studies

Country (Reference)	Total HBCD dietary exposure (ng/kg bw/d)			
	Child			Adult
	1–6 months	2 years	12 years	
UK (FSA, 2006)		**24** (1.5–2.5 years, average consumer) **50** (1.5–2.5 years, high-level consumer)	**6** (11–14 years, average consumer) **12** (11–14 years, high-level consumer)	**5.9** (average consumer) **12** (high-level consumer)
European dietary surveys (EFSA, 2011)	**0.6 -142** (breast-fed infants, average consumer) **0.9 -213** (breast-fed infants, high consumer)	**0.15 – 1.85** (other children, average consumer) **0.8 – 4.46** (other children, high-level consumer)	**0.09 – 1.06** (adolescents, average consumer) **0.47 – 2.68** (adolescents, high-level consumer)	**0.09 – 0.99** (average consumer) **0.39 – 2.07** (high-level consumer)
The Netherlands (de Winter-Sorkina et al., 2003)				**1.5** (lower bound intake) **2.9** (medium bound intake)
China (Shi et al., 2009)				**0.43** (medium bound intake) **0.6** (upper bound intake)
Belgium (Goscinny et al., 2011)				**0.99**
Sweden (Törnkvist et al., 2011)				**0.14**

As the studies presented used differing methodologies to estimate dietary exposure, the results from these studies cannot be compared directly to each other. It can be seen from the table that the UK estimates gave the highest HBCD dietary intakes.

Collection of the food consumption data in the UK and Australia used similar methods, with both the UK's National Diet and Nutrition Survey and Australia's National Nutrition Survey having conducted dietary recall interviews and collected self-completion questionnaires in accordance with internationally accepted principles (WHO, 2009). In particular, the UK study gives estimates for all of the age groups considered in this assessment apart from infants (1–6 months) for which exposure is considered to be via breast milk. Thus, the exposure estimate from FSA (2006) is chosen with the "average consumer" and "high-level consumer" values taken as the typical and reasonable worst-case intake estimates, respectively, of HBCD from food in Australia.

5.3.5 Breast milk

HBCD has been on the world market since the 1960s. Use in insulation boards started in the 1980s (POPRC, 2011). Human breast milk data from the 1970s to 2000 show that HBCD levels have increased since HBCD began to be widely used as a brominated flame retardant in the 1980s.

No Australian study of HBCD concentrations in breast milk has been reported. Two studies have examined the change in HBCD concentrations in breast milk over time (Table 5.6) – 1 in Sweden (Fangstrom et al., 2005) and 1 in Japan (Kakimoto et al., 2008). Both examined the level of HBCD in pooled breast milk, with pooled milk generally from between 13 and 35 individuals, although the 1980 and 1984 pools from Sweden were taken from over 100 individuals each. In the Japanese study, analysis was normally restricted to mothers in the age range 25 to 29, but separate pools with mothers over 30 were investigated from 2003 to 2006 (referred to as "pool 2" in the Japanese results in Table 5.6). The results did not show a consistent pattern of increase or decrease of HBCD concentration with maternal age. In both countries, the concentration of HBCD in breast milk was low before 1988 and appears to have reached a plateau value since this time. There are indications of a possible decrease in Sweden since 2002.

Table 5.6. Time series HBCD levels in lipid component of human breast milk in Sweden and Japan

Year	Subjects	Mean HBCD levels (ng/g lw)	Year	Subjects	Mean HBCD levels (ng/g lw)
Sweden			*Japan*		
1980	116	0.084	1973	21	n.d.
1984	102	0.096	1978	32	n.d.
1988	20	0.24	1983	20	n.d.
1990	20 (pool 1) 20 (pool 2)	0.22 0.20	1988	31	0.43
1992	20	0.29	1993	33	2.5
1994	20	0.38	1998	35	1.6
1995	20	0.51	1999	30	1.4
1996	20	0.33	2000	27	1.8
1997	20	0.29	2001	31	1.4
1999	20	0.37	2002	28	1.0
2001	20	0.54	2003	20 (pool 1) 30 (pool 2)	2.1 2.0
2002	20	0.60	2004	13 (pool 1) 26 (pool 2)	1.0 2.3

2003	15	0.49	2005	20 (pool 1) 21 (pool 2)	1.5 1.6
2004	20	0.39	2006	25 (pool 1) 25 (pool 2)	4.0 1.4

lw = lipid weight; n.d. = not determined

A majority of HBCD measurements in breast milk internationally since 2000 (Table 5.7), have shown mean HBCD concentrations in a range of 0.5–2 ng/g lw. Where individual sampling has been undertaken and medians have been reported, these are generally lower than mean or pooled results, in the range 0.3–0.4 ng/g lw. Due to the consistency of results between developed countries across Europe, Asia and North America, it is considered likely that the international data will be representative of Australian breast milk levels of HBCD.

Table 5.7. HBCD levels in lipid component of human breast milk from overseas studies

Country	Subjects	Sampling date	HBCD level (ng/g lw) Mean (Median)	HBCD level (ng/g lw) Range	Comment	Reference
North America						
Texas	9	2002	0.5 (0.5) ug/kg	0.2-0.9 ug/kg	Result for α-HBCD only	Ryan et al., 2006
Canada	8	2002–03	3.8 (1.6) ug/kg	0.4-19 ug/kg	Result for α-HBCD only	Ryan et al., 2006
Mexico	7	Not stated	2.1	0.8–5.4		Lopez et al., 2004
Europe						
Murmansk, Russia	14	2000	0.47 (0.45) ug/g	0.20–1.15 ug/g	HBCD detected in 8 of the 14 samples (detection limits ranged between 0.012 and 0.034 ng/g ww)	Polder et al., 2008b
Arkhangelsk Russia	23	2002	0.71 (0.62) ug/g	0.24–1.67 ug/g	HBCD detected in 3 of the 23 samples (detection limits ranged between 0.012 and 0.034 ng/g ww)	Polder et al., 2008b
Goteborg, Sweden	33	2001	0.45 (0.3)	0.15–2.37	21 samples were below the LOD (15 pg/g milk)	Aune et al., 2001
Norway	9		ND	0.25–2.0	The women lived near a municipal waste incinerator	Thomsen et al., 2003
Norway	9	2001–09	(0.27)	<LOQ – 3	Detected in 32/71 samples (LOQ~0.2 ng/g lipid wt)	Thomsen et al., 2010
Norway	10	2000–01	0.13	ND	Only 1 of the 10 samples was positive	Polder et al., 2008a
Norway	193	2003–06	1.10 (0.54)	0.10–31	Each participant provided a pool sample made up of samples taken over 8 consecutive days	Eggesbo et al., 2011
Uppsala, Sweden	30	2002–03	0.42 (0.35)	0.16–1.5	6 samples were below the limit of detection (0.2–0.37 ng/g lipid wt)	Lignell et al., 2003
Sweden	5	Not stated	1.1	0.3–3.2		Lopez et al., 2004
Uppsala, Sweden	29	2004	0.58 (0.33)	0.14–4.36	All samples were above the limit of detection (0.07–0.17 ng/g lipid wt)	Lignell et al., 2005
Belgium	197	2006	1.5		Women between 18 and 30 years old distributed over	Colles et al., 2008

					all Belgian provinces	
Spain	33	2006–07	47 (27)	3–188	HBCD detected in 30/33 samples with levels of diastereoisomers determined and body burden of mothers and infant exposure reported	Eljarrat et al., 2009
United Kingdom	34	Not stated	5.95 (3.83)	1.04–22.37	HBCD was detected in all samples	Abdallah & Harrad, 2010
Uppsala, Sweden	92	2000–04	(0.3)	<0.2–4.4	77% >LOQ	Glynn et al., 2011
Gothenburg, Sweden	36	2001	(<0.4)	<0.4–2.4	39% >LOQ	Glynn et al., 2011
Lund, *Sweden	36	2003	(0.4)	<0.2–5.9	93% >LOQ	Glynn et al., 2011
Lycksele, *Sweden	39	2003–04	(0.4)	0.09–10	100% >LOQ	Glynn et al., 2011
Asia						
China	1237	2007	1.21 (rural); 0.86 (urban)	0–2.78	Results for α-HBCD only (Samples collected from 12 provinces of China from primiparous mothers)	Shi et al., 2009
Philippines	33	2004	0.86 (0.62)	0.13–3.2	ΣHBCD. 22/33 samples were from mothers living at periphery of dumping site, 11/33 from reference site	Malarvannan et al., 2009
Vietnam – Hanoi	9 (office workers, housewives)	2007	(0.33)	0.070–1.4	Reference group	Tue et al., 2010
Vietnam – Dong Mai	4 (recycler, farmers)	2007	(0.42)	0.11–0.97	e-waste recycling site (batteries)	Tue et al., 2010
Vietnam – Trang Minh	11 (recyclers, housewives)	2007	(0.38)	0.11–3.3	e-waste dismantling site	Tue et al., 2010
Vietnam – Bui Dau	5 recyclers	2007	(2.0)	1.4–7.6	e-waste dismantling site	Tue et al., 2010
Vietnam – Bui Dau	4 non-recyclers	2007	(0.36)	0.29–1.2	e-waste dismantling site	Tue et al., 2010

ND = Not detected

The studies appear to be exhibiting log normal distribution. For a log normal distribution with <50% non-detects, the median value is not affected and there is minimal impact on the mean value, as this is predominantly determined by the small number of very high individual values.

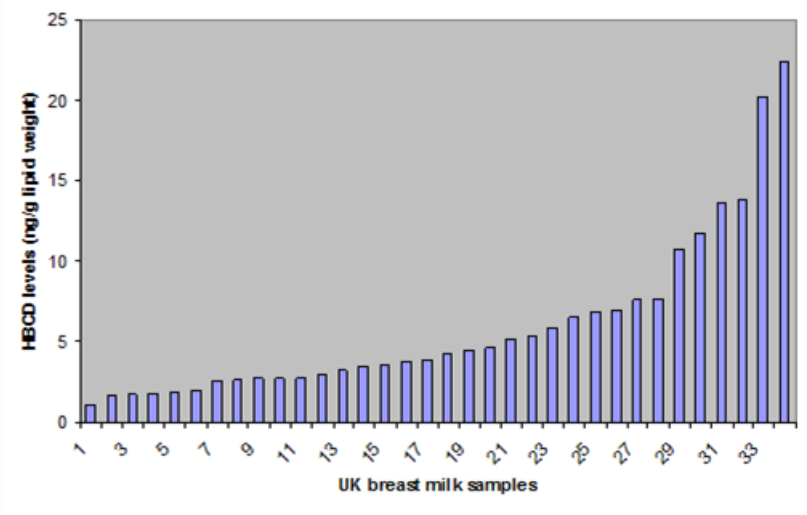


Figure 5.2. HBCD concentrations in breast milk from Abdallah & Harrad (2010).

The HBCD levels in breast milk investigation by Abdallah and Harrad (2010) in the UK were plotted and are presented in Figure 5.2. This study has the largest number of samples for which individual values were available. The levels appear to demonstrate a log normal distribution similar to the individual dust samples.

Further analysis of the samples gave mean, median, 75th percentile, and 95th percentile values of 6.0, 3.8, 6.9, and 16.0 ng/kg lipid weight. In taking into account the high variability of the results, the 75th percentile reasonably well represents the range of HBCD concentrations in Table 5.7 and is chosen as the typical level. The 95th percentile HBCD concentration in breast milk analysed from the Abdallah and Harrad (2010) study is chosen as the reasonable worst-case value. This value is within the range seen in several other studies of HBCD concentrations in breast milk (Table 6.7) in the Western Hemisphere countries.

Breastfeeding in Australia

The 2006–2007 Longitudinal Study of Australian Children showed that 88% of children were being fully breastfed (i.e. breastfeeding with no other food or fluid intake) at discharge, and 56% 3 months later (AIFS, 2008). The rate of full breastfeeding declined to 46%, 28%, and 14% at 4, 5 and 6 months, respectively. The Australian Health Ministers' Conference (2009) reported that Australia is relatively comparable with other OECD countries in breastfeeding up to 3 months. In this report, a fully breastfeeding duration of 6 months is selected as a conservative approach for exposure estimation.

The daily intake of breast milk by infants in Australia is not available. The NHMRC (2003) has recommended average intakes of fully breastfed infants at 710 mL/d for zero to 2 months and 800 mL/d for 3 to 8 months. A higher volume of 850 mL/d is also cited in the WHO report (WHO, 1985). The *Child-specific exposure factors handbook* from US EPA indicated that the weighted average daily breast milk intake of breast milk for infants from 1 to 6 months in age ranges from 702 to 765 mL/d, and the upper percentile values (mean plus 2 standard deviations) for the same age groups, 1007 to 1059 mL/d (US EPA, 2002), and these figures are adopted for oral exposure estimation via breast milk in infants. The US EPA recommendations are based on the weighted averages of several studies and are similar to the values reported for Swedish infants (ECETOC, 2001).

Estimation of oral exposure

Based on the weighted average and upper percentile intake volumes and bodyweights (US EPA, 2002), the bodyweight adjusted milk intake rates for the typical case and the reasonable worst-case of infants aged 1 to 6 months are 139 g/kg bw/d and 194 g/kg bw/d, respectively. The bodyweights for infants (one to 6 months) in the *Child-specific exposure factors handbook* (US EPA, 2002) and used in this assessment are comparable to the Australian data published by Gracey and Hitchock in 1985 (Lester, 1994). Details of estimation of oral exposure via breast milk for infants aged 1 to 6 months are shown in the Appendix 3, Scenario 4.

Table 5.8 shows the HBCD levels through oral exposure via breast milk in infants for both typical scenario and the reasonable worst-case scenario. The exposure is estimated based on the following additional assumptions:

- Density of breast milk is 1.03 g/mL.
- Lipid content of breast milk is 4%.
- 100% HBCD ingested is absorbed.

Table 5.8. Estimation of HBCD levels from oral exposure via breast milk in infants aged 1 to 6 months

	HBCD (ng/g lw)	Lipid content	Intake rate (g/kg bw/d)	Bioavailability	Oral exposure (ng/kg bw/d)
Typical case	6.9	4%	139	100%	38
Reasonable worst-case	16	4%	194	100%	124

Exposure to HBCD from consumption of breast milk in children is estimated in the fully breastfed age group of infants aged 1 to 6 months. Exposure level from the reasonable worst-case scenario is at least 3 times the typical exposure scenario level.

5.4 Summary of public exposure estimates

The public exposure estimates from all sources are summarised in Table 5.9.

Table 5.9. Summary of daily exposure of the public

	Typical value and reasonable worst-case HBCD exposures (ng/kg bw/d)			
	Infants (1–6 months)	**Toddlers (2 years)**	**Children (12 years)**	**Adults**
Exposure from consumer products				
Oral	Negligible	Negligible	Negligible	Negligible
Inhalation	Negligible	Negligible	Negligible	Negligible
Dermal	0.04 (w-c)	0.04 (w-c)	0.03 (w-c)	0.03 (w-c)
Exposure from the environment				
Total indoor exposure	0.04 (typ) 0.28 (w-c)	35 (typ) 231 (w-c)	4.9 (typ) 32 (w-c)	2.0 (typ) 13 (w-c)
Total outdoor exposure	0.002 (w-c)	4.8 (w-c)	0.7 (w-c)	0.3 (w-c)
Food consumption	Negligible	24 (typ) 50 (w-c)	6 (typ) 12 (w-c)	5.9 (typ) 12 (w-c)
Breast milk consumption	38 (typ) 124 (w-c)	Not estimated	Not estimated	Not estimated
Total	**38 (typ) 124 (w-c)**	**59 (typ) 286 (w-c)**	**11 (typ) 45 (w-c)**	**8 (typ) 25 (w-c)**

typ = typical; w-c = reasonable worst-case

Release, into indoor air, of HBCD from consumer products is the major contributor to the internal exposure when compared to the levels of HBCD in the general environment. Toddlers (2 years) are the most exposed subpopulation from HBCD exposures because of their greater tendency for hand-to-mouth behaviour as well as a relatively greater food intake per bodyweight than other age groups.

The HBCD internal exposure estimates from dust and breast milk were based on the assumption that the distribution of the HBCD levels are log normal as compared to a normal distribution. The large difference between the typical and reasonable worst-case estimates is consistent with the expected exposure patterns for a chemical with log normal distribution in exposure media.

5.5 Biological monitoring data

Biological monitoring provides a means to assess exposure and health risk to the public. It entails measurement of the concentration of a chemical determinant in the biological media (for example, blood or urine) of those exposed and is an indicator of the uptake of a substance. Biological monitoring can assist in the determination of body burden and in detecting past exposure where chemicals are eliminated slowly.

HBCD has been detected in human adipose tissue, milk and blood (Covaci et al., 2006; Johnson-Restrepo et al., 2008; Arnot et al., 2009). General population exposure to HBCD has been attributed to its presence in food (van Leeuwen and de Boer, 2008), outdoor air and indoor air (Law et al., 2008b) and indoor dust (Covaci et al., 2006; Roosens et al., 2009).

The Roosen et al. study (2009) examined the relationship between the body burden of HBCD and exposure via 2 pathways (food and dust) for adults. The authors measured HBCD levels in the blood serum of 16 Belgian adults and compared them with their dietary intake collected over one week, as well as dust samples from their bedrooms.

Duplicate diet samples (n = 165) were collected between May and June 2007. To collect dust samples, 4 square metres of bare floor in the participants' rooms were vacuumed for 4 minutes. Samples were collected using nylon sampling socks (25 μm mesh) mounted in the furniture attachment of the vacuum cleaner. A 10 mL blood sample was collected from each participant for HBCD anaysis. All dust samples were analysed for HBCD by LC-MS/MS.

Only 13 of 165 duplicate diet samples contained concentrations of HBCDs above LOQ, with concentrations ranging between 0.01 and 0.35 ng/g ww (average, 0.13 ng/g ww). HBCD was detected in all dust samples and ranged between 33 and 758 ng/g dw (mean, 160 ng/g dw; median, 114 ng/g dw).

To estimate exposures via dust ingestion, the average adult dust ingestion rate of 20 mg/d and the high dust ingestion rate of 50 mg/d were multiplied by the concentrations of HBCD detected in dust from the rooms of individual participant. This yielded exposures of 1.1–15 ng HBCD/d (mean, 3.2 ng for an average dust ingestion rate) and 2.8–38 ng HBCD/d (mean, 8.0 ng for a high dust ingestion rate). The total intake of HBCD for individual participants was calculated as the sum of dust ingestion and dietary intake.

Serum HBCD levels were in the range of <0.5–11 ng/g lw (lipid weight) (mean, 2.9 ng/g lw). Seven of 16 blood serum samples were below LOQ.

To examine the relationship between exposure and serum concentrations of HBCD, bl concentrations of HBCD were plotted against exposure a) via diet and dust; b) via diet alone; and c) via dust ingestion alone. No significant correlation was observed between serum concentrations and intake via diet alone or combined food and dust exposure. HBCD concentrations in serum significantly correlated with estimates of exposure via dust (r_s = 0.86). The influence of dust ingestion on the serum concentrations was attributed to it being a relatively constant exposure in contrast to dietary intake, which is influenced by irregular spikes in exposure through occasional ingestion of more highly contaminated food.

A number of other studies have been conducted to determine the levels of HBCD in human blood and breast milk samples. Results of the breast milk studies are summarised in Tables 5.6 and 5.7, and blood studies are summarised in Table 5.10. The highest measured serum concentrations of HBCD were found in 2 studies which looked at groups with expected higher exposures than the general public – one on occupationally exposed workers (Thomsen et al., 2007) and the other on people who ate fish from a lake known to be polluted with brominated flame retardants (Thomsen et al., 2008).

The linear one-compartment open pharmacokinetic model described by Geyer et al. (2004), discussed in Section 8.1, is used to estimate public exposure from the biological monitoring data. The method and equation used in the estimation can be found in Appendix 3, Scenario 5. Using a terminal elimination half-life of 64 d and a lipid mass of 16.1 kg per person (the mean of male and female data), the level of 1.1 ng/g lw in serum (from the study of Weiss et al., 2004) for a 60 kg bodyweight person corresponds to a HBCD daily intake of 3.2 ng/kg bw/d. Based on the maximum HBCD level of 7.0 ng/g lw in adult serum from the same study, the daily HBCD exposure is estimated to be 20 ng/kg bw/d. Uncertainties in the estimation include the values of estimated elimination half-life and the human lipid mass. In addition, the metabolic processes of HBCD and the HBCD metabolites are not included in the estimation.

Table 5.10. Summary of human serum or cord plasma biological monitoring studies of HBCD

Location	Study subjects	N	Blood origin	HBCD concentration (ng/g lw)		Reference
				Mean (Median)	Range	
The Netherlands	90 pregnant women (8 samples from pregnancy week 20; 70 samples from week 35)	78	serum	1.1 (1.3)	<0.16–7.0	Weiss et al., 2004
		12	cord	2.4 (0.32)	<0.16–4.2	
Mexico	5 women from an urban area	5	serum	1.2	0.7–2.5	Lopez et al., 2004
Norway	Occupational exposure	10	serum	(101)	6–856	Thomsen et al., 2007
Norway	Fish eaters – men	41	serum	9.6 (4.1)	<1–52	Thomsen et al., 2008
	Fish eaters – women	25	serum	3.7 (2.6)	<1–18	
Sweden		50	serum	(0.5)	0.24–3.4	Weiss et al., 2006
Belgium		16 (+7)	serum	2.9 (1.7)	<0.5–11	Roosens et al., 2009
The Netherlands	Health volunteers ages 19–78, 48 males and 43 females	91	serum	(197 pg/g serum*)	96–356 pg/g serum*	Peters, 2004
Greece	30 samples from clerks working full-time with computers and 31 samples from control group with no computer use, ages 20–65	61	serum	3.39 (1.32)	0.49-38.8	Kalantzi et al., 2011

Europe	47 European MPs	47	serum	0.063 ng/g whole blood	n.a.	World Wildlife Fund, 2004

* Peters (2004) stated that pg/g serum can be converted to pg/g lipid by multiplying the values by 150.

Taking into account these uncertainties, the calculations based on biomonitoring results can be compared with the deterministic dose estimates in Table 5.9. The estimates of total exposure to the indoor air environment for the adult general public show a close similarity to the biomonitoring results. Biomonitoring results for children are not available.

Reporting of monitoring data is complicated by the observation that the distribution of individual results covers orders of magnitude in concentrations, as observed for HBCD dust levels (Table 5.2) and human breast milk (Table 5.7). The distribution of results for HBCD concentrations in breast milk has a log normal distribution similar to the values of HBCD in dust, as evident in Figures 1 and 2. While no study reported individual serum data, the biomonitoring results could very well exhibit the same distribution since there is a consistent pattern of having a wide range of values as well as the mean having a larger value than the median in most, if not all, of the studies reported.

6. Occupational exposure

6.1 Methodology for assessing exposure

Australian industries import a range of commercial grade HBCD for further formulation for industrial uses and HBCD containing products in the form of articles. Occupational exposure may occur during transport, storage, repacking, formulation of products, treatment of textiles, plastic manufacture and/or use of end products containing HBCD.

In the assessment of occupational exposure, it is preferable to use measured data. The use of measured data is in accordance with international methods (WHO/IPCS, 2000) wherein the 50th percentile (median) and the 90th percentile values are the reported occupational exposure levels. In the occupational exposure assessment for HBCD, the median and the 90th percentile values are used to represent the typical and reasonable worst-case estimates, respectively.

Depending on the availability of appropriate measured data, internal exposure levels for the established scenarios are derived from either measured or modelled data.

In the absence of measured data for certain worker scenarios, modelled data are used in this assessment. In modelling exposures, the Estimation and Assessment of Substance Exposure (EASE) model is used. The EASE model was developed by the UK Health and Safety Executive (HSE) and is a general-purpose predictive model for workplace exposure assessments. Daily exposures are predicted as a range of exposure values derived from databases of measured exposures from workplace environments based on a standard 8 h working day (EC, 2003). The model considers dermal and inhalation exposures to substances, which may be present as solids, liquids, gases or vapours. EASE is able to consider a wide range of workplace activities, including maintenance and sampling procedures, and can also account for some risk management measures (e.g. use of low dust formulation or local exhaust ventilation). It is acknowledged that the EASE model takes a conservative approach and is likely to overestimate exposure (Creely et al., 2005).

The EASE model assumes that the operator spends a full shift (8 h) working at different sites and is exposed to the raw HBCD. Since the majority of work processes involving potential exposure to HBCD do not fit this assumption, the estimates need to be adjusted based on each use scenario and process description.

6.2 Routes of exposure

During occupational use of HBCD powder, granules, dispersions and HBCD-containing end-use products, the main exposure routes are dermal and inhalation, though ocular exposure may also occur. Occupational exposure by the oral route is unlikely under normal circumstances. However, accidental ingestion may occur due to hand contamination with HBCD. Oral exposure may occur from ingestion of non-respirable particles deposited in the upper respiratory tract following mucociliary clearance.

6.2.1 Inhalation route

The evaporation of a chemical is dependent on its vapour pressure at both the ambient temperature and the temperature during use. Under standard conditions, HBCD is a solid with a low vapour pressure (6.3×10^{-8} kPa at 21 °C) and high melting point (>170 °C). The vapour pressure of HBCD is not expected to lead to appreciable workplace vapour concentrations at higher temperatures in industrial uses. Inhalation exposure to HBCD vapour is considered to be low.

Solid commercial grade HBCD available on the market can be divided into 3 grades according to their particle sizes. While the distribution pattern of particle sizes may vary from batch to batch, a range for the mean sizes of each grade was supplied by industry:

- fine-grade powder (mean size 2–19 □m)
- standard-grade powder (mean size 20–150 □m)
- granular grade (mean size 560–2400 □m).

The particle size distribution has a significant effect on the degree of inhalation exposure, as it determines the degree of dust generation and the fraction of particles that are inspirable. Particles less than 10 □m in diameter are respirable and can reach the alveolar regions of the lung, where they are able to be absorbed. Particles between 10 □m and 180 □m are non-respirable and are deposited in the upper respiratory tract and transported to the pharynx and swallowed.

The importers of HBCD indicated that fine-grade HBCD powder is not used in Australia. Only standard-grade powder and granules of HBCD have been used. Recent information has indicated that the use of standard-grade powder has now been discontinued. Industry reported that 3 standard-grade HBCD powders were available in the Australian market that contained 50%, 75% or 100% particles less than 100 μm in diameter. Three granular grades of HBCD are used by Australian industries. The 3 granular products have approximately 1%, 5% and 10% of the particles, respectively, with a diameter less than 100 □m. Inhalation exposure via airborne dust in the workplace is possible when workers handle either the standard-grade powder or granular grades of HBCD.

6.2.2 Dermal route

Dermal exposure is possible when workers handle HBCD liquid formulations, powder or granules. Dermal absorption following exposure to HBCD granules is expected to be less than that of the powder form, since granules are unlikely to adhere and remain on the surface of the skin for a prolonged period.

Based on an in-vitro dermal absorption study in human breast skin, the dermal absorption rate was determined to be 4% for liquid and powder products, and 2% for granules (Roper et al., 2005).

Following incorporation of HBCD into the polymeric matrix or textile fibres, HBCD is not bio-available via the dermal route. Very small amounts of HBCD may be available at the surface of the articles due to leaching or blooming. However, the dermal exposure from contact with articles is expected to be very low.

6.3 Exposure modelling

6.3.1 Exposure scenarios

The exposure scenarios selected were based on the use pattern of HBCD in different groups of workers. The workers that may potentially be exposed to HBCD are grouped by industry. These are listed in Table 6.1. Some of the acivities listed below such as weighing and repacking powdered HBCD are not being undertaken currently in Australia, , however exposure for these activities was also calculated as these activities may resume unless regulated.

Individual exposure assessments are undertaken for the groups of workers which are expected to have the highest exposure within the industries, as indicated by asterisks.

Table 6.1. Occupational groups based on industry

Importation and repackaging	Wharf operators Forklift and truck drivers Storage workers Repackers*
Polymer industry	Compounding and conversion machine operators* Moulding machine operators* Laboratory analysts Formulators Quality controllers Packers Storage and transport workers Cleaners Maintenance workers Waste disposal contractors
Textile industry	Formulators* Textile coating workers* Laboratory analysts Quality controllers Packers Storage and transport workers Cleaners Maintenance workers Waste disposal contractors
Electric and electronic industry	Assembly line workers Quality controllers Packagers Repairing and maintenance workers Electricians
Furniture industry	Cutters* Sewing machine operators Upholstery workers*
Building industry	Builders Insulation installers*
Other industries	Office workers Taxi drivers Other automobile drivers Airplane crews

The various use patterns for which occupational exposures are assessed are grouped into 4 scenarios based on the nature of the work and the HBCD formulations:

- **Importation and repackaging:** workers handling the imported commercial grade HBCD (powder) may have high occupational exposure during repackaging into smaller pack sizes or in the event of an accident or spill.
- **Polymer industry:** workers in the polymer industry handle solid forms of HBCD in weighing and compounding, and plastic products containing HBCD in conversion and moulding.
- **Textile industry:** the formulators in the textile industry use liquid HBCD concentrates to formulate dispersions, and other workers use the HBCD-containing dispersions to coat fabrics.
- **End users of HBCD-containing products/articles:** all workers handling semi or end products containing low concentrations of HBCD – for example, electricians, upholsterers, builders and other industry workers in the office and transport industry – are considered as end users.

6.3.2 Assumptions used in estimating exposures

The following assumptions were used in exposure modelling:

- HBCD powder products have 100% particles less than 100 μm (used as the worst-case scenario for inhalation exposure estimation).
- HBCD granular products have 10% particles less than 100 μm (used as the worst-case scenario for inhalation exposure estimation).
- Maximum concentrations of HBCD are 100% for powder and granular formulations, and 60% for liquid formulations (Section 4).
- Bodyweight of an average worker = 70 kg.
- Respiratory rate for average workers = 1.3 m^3/h (EC, 2003).
- Respirable/inhalable fraction = 1 (EC, 2003).
- Surface area of hands and forearms for dermal exposure = 1000 cm^2 (US EPA, 1997).
- Surface area of hands = 840 cm^2 (US EPA, 1997).
- Inhalation absorption rate = 100%.
- Dermal absorption for HBCD liquid and powder = 4%.
- Dermal absorption for HBCD granule = 2%.

Exposure was estimated based on typical and worst-case scenarios with the worker not using personal protective equipment (PPE).

6.4 Exposure during importation and repackaging

Solid forms of HBCD, either the powder/granule HBCD or EPS resin containing HBCD, are imported in polylined paper bags, which are shrink-wrapped in plastic and placed on pallets. Liquid dispersions containing HBCD are imported in high-density polypropylene drums. Exposure during importation and transportation is likely to be negligible, except in cases of breached packaging.

One company in Australia repackaged the imported 25 kg bags of HBCD granules or powder into 15 kg bags. This operation involved a single worker working for 2 h a day. One tonne of HBCD was repackaged approximately every 3 months over a period of 5 d. The repackaging was an open or semi-closed process. Controls reported to be in place included a dust extraction system and the use of PPE, including gloves and a dust mask. Repackaging workers were expected to be one of the groups with high exposure potential through both inhalation and dermal routes.

6.4.1 Measured data

No measured data in Australia and overseas are available in the importation and repackaging process. Overseas measured data for addition and weighing in the polymer industry (Section 6.5.1) are applicable to Australian workplaces to a large extent due to the similarities of many of the tasks.

6.4.2 Modelled exposures

Inhalation exposure during repackaging

The EASE operation description used is dry manipulation and the description of the solid used is non-aggregating, non-fibrous with a particle size in the inhalable range. The full shift concentration is predicted to be 2-5 mg/m^3 with local exhaust ventilation (LEV) and 5–50 mg/m^3 without LEV. The EASE-predicted range with LEV is used to represent a typical HBCD workplace concentration while the EASE-predicted range without LEV is used as the worst-case HBCD workplace concentration.

The internal exposure via the inhalation route is estimated using the assumptions in Section 6.3.2 and Equation 1:

$$I_{inh} = \frac{f_{resp} \cdot C_{air} \cdot V_{air} \cdot t \cdot \frac{A_{inh}}{100} \cdot cf}{BW} \qquad \text{Equation 1}$$

where: I_{inh} = internal exposure of HBCD via the inhalation route, in μg/kg bw/d

f_{resp} = respirable/inhalable fraction of HBCD, dimensionless

C_{air} = HBCD concentration in the air estimated using EASE, in mg/m^3

V_{air} = ventilation rate of worker, in m^3/h

t = duration of exposure per day, in h/d

A_{inh} = inhalation absorption rate, in %

cf = conversion factor, 1000 μg/mg

BW = bodyweight of worker, in kg.

The average time spent by a worker repackaging HBCD powder is 2 h a day. With other assumptions listed in Section 6.3.2 and using the EASE estimates as HBCD powder concentrations in the air, the internal inhalation exposures with and without LEV are 74–186 μg/kg bw/d and 186–1860 μg/kg bw/d, respectively. The exposure will be lower if the worker does not carry out repackaging tasks on a daily basis during the production period.

For HBCD granules, the air concentration of HBCD is 10% of the EASE estimates for repackaging HBCD standard powder products according to the assumption in Section 6.3.2. Thus, the typical concentration during the repackaging of HBCD granules is 0.2–0.5 mg/m^3 and the worst-case concentration is 0.5–5 mg/m^3. Based on these concentrations, the internal exposures via inhalation with and without LEV were in the ranges 7.4–18.6 μg/kg bw/d and 18.6–186 μg/kg bw/d, respectively.

Dermal exposure during repackaging

The EASE operation description used is direct handling and intermittent, non-dispersive use. The predicted external dermal dose is 0.1–1 mg/cm^2/d.

The internal exposure via the dermal route is estimated using the assumptions in Section 6.3.2 and Equation 2:

$$I_{derm} = \frac{E_{EASE} \cdot \frac{C}{100} \cdot S_{derm} \cdot \frac{A_{derm}}{100} \cdot cf}{BW} \qquad \text{Equation 2}$$

where: I_{derm} = internal exposure of HBCD via the dermal route, in μg/kg bw/d

E_{EASE} = external dose estimated by EASE, in mg/cm^2/d

C = concentration of HBCD, in %

S_{derm} = surface area of exposed skin, in cm^2

A_{derm} = dermal absorption rate, in %

cf = conversion factor, 1000 μg/mg

BW = bodyweight of worker, in kg.

During repackaging of HBCD standard powder products, internal exposure from dermal contact is in the range 57–570 μg/kg bw/d. During repackaging of HBCD granules, the internal exposure is in the range 29–290 μg/kg bw/d.

6.5 Exposure in the polymer industry

HBCD has been used in the polymer industry as an additive to impart flame-retardant properties to polystyrene foam, polystyrene articles and polypropylene articles. Workers in the polymer industry have been exposed to HBCD during compounding, conversion or moulding activities. The occupational activities involved in this industry are described below.

6.5.1 Compounding raw HBCD into resins

The compounding processes are usually semi-automated. Exposure is more likely during manual operations such as weighing and/or adding HBCD to the reactor, packing the compounded product, taking samples from the process, and cleaning and maintenance of the equipment.

Three Australian companies provided information for the assessment on compounding the raw HBCD solid into polymeric resins, including EPS resin, polystyrene or polypropylene masterbatch pellets. The final concentration of HBCD is <1% in the EPS resin and 2–50% in the masterbatch pellets.

Weighing and manual addition of the HBCD to the reactor are the only tasks involving raw HBCD, and are therefore considered to be the tasks likely to have the highest inhalation and dermal exposure. Both powder and granular forms of HBCD have been used in Australia. Table 6.2 details the various HBCD formulations, exposure duration, frequency, industrial controls and PPE used during the processes of compounding polymeric resins containing HBCD in Australia. These data are obtained from descriptions provided by applicants.

The weighing of HBCD prior to addition to the masterbatch was reported to occur at only 1 site in Australia. At the other 2 sites, HBCD is added directly from the 25 kg packed bags into the chute. The operation involves manually lifting the bags to the charge chute, cutting them open and pouring the contents into the chute.

6.5.2 Conversion of polymeric resin into EPS foam products

The flame-retarded EPS resin with HBCD is converted into products such as EPS foam boards or moulded packaging materials. The major use of EPS foam products is in the building industry. Manufacture of EPS foam products is conducted in 2 stages: prefoaming and moulding.

In the prefoaming stage, EPS resin is expanded to form beads. This prefoaming process is completely automated in Australia. At this stage, concentrations of HBCD in EPS beads are low (<1%), the bead size is not in the inhalable range and HBCD is incorporated into the polymeric matrix. Therefore, workplace exposure to HBCD is expected to be low.

The second stage refers to the moulding of the prefoamed beads into blocks or other designated shapes, which is usually a semi-automated process in Australia. For the same reasons as discussed above for the prefoaming stage, occupational exposure to HBCD is expected to be low during the moulding process.

The EPS foam blocks are cut into sheets or customer-required shapes if necessary. This cutting can be carried out by machine using sawing or hot wire cutting, or by handsaw. The off-cuts are granulated and recycled within the plant. Applicants have advised that the cutting task will not result in the formation of inhalable dust, as the particles generated are too large. Dermal contact with blocks, sheets or other moulded foams occurs during manual handling. However, HBCD is incorporated into the foam matrix and is not bio-available. Considering these factors as well as the low concentration of HBCD in the foam (<1%), this cutting task is likely to result in low exposure to HBCD.

Table 6.2. Occupational exposure scenarios from compounding sites in Australia

Work process	HBCD formulations (mean particle size)	Task	Number of workers	Exposure duration (min/day)	Exposure frequency (days/year)	Industrial controls	Personal protective equipment
Expandable polystyrene resin compounding	Granules (2 mm) Powder* (50–60 μm)	Adding HBCD to the mixture	1	10	180	Local exhaust ventilation	Overalls, safety glasses, leather or chemical resistant gloves, dust masks and respirators (optional)
Polypropylene masterbatch compounding	Powder (50–60 μm)	Weighing and adding HBCD to the mixture	1	30 (weighing) 20 (addition)	3**	Ventilation in the weigh room Dust extraction at all weighing points and the blender charge hatch	Overalls, gloves, respirators fitted with SR510 particle filters and 315 gas filters
Polystyrene masterbatch compounding	Powder (50–60 μm)	Addition	1	15	1	Dust extraction unit	Dust mask, safety glasses

* Only used in the past
** No production runs since May 2004.

6.5.3 Injection moulding to produce plastic articles

In the injection moulding process, the masterbatch pellets (containing the polymer and HBCD, along with any other additives) are heated to create a molten plastic, which is then injected under high pressure into a steel mould. After cooling, the mould is opened and the solid plastic shape is removed.

One Australian company uses the injection moulding method for the manufacture of exhaust fan blades from a polypropylene masterbatch containing HBCD at <25%.

The injection moulding process is usually designed to be a closed or semi-closed system. Manual handling of the masterbatch pellets or finished products may occur. As these materials have HBCD encapsulated in the polymer matrix and are of large particle size, no significant dermal or inhalation exposure to HBCD is expected.

6.5.4 Measured data

No measured data on Australian worker exposures in the polymer industry was provided. Limited overseas information (from Europe) on actual concentrations of HBCD in this industry is available. In the absence of sufficient Australian information, the European studies are considered similar to the Australian setting and these measured data are used in calculating internal exposures to HBCD.

Inhalation exposure during addition and weighing

HBCD powder

Searl and Robertson (2005) quantified the airborne concentrations of HBCD in different working environments, including during manual addition of HBCD powder of unspecified grade to a reactor for the synthesis of EPS resin. In this study, respirable and non-respirable concentrations of HBCD in the air were determined. Personal samples were collected by samplers specially designed by Scotland's Institute of Occupational Medicine, and analysed by HPLC. Four plants were involved in the study and the generation of HBCD dust was found to be highly variable, depending on how the task was carried out and the environmental settings.

HBCD was added to reactors each time a batch of EPS was produced in the plants. There were usually 1 to 2 batches per shift, but in one plant there were 3 or 4 batches during a shift. The task took 10 to 15 minutes, of which 5 minutes involved handling HBCD. In one plant, the sacks were split and weighed concurrently. In other plants, bags of HBCD were weighed one to 3 weeks in advance. During weighing, HBCD powder was transferred between sacks using a plastic scoop. Weighing and transfer of HBCD took about an hour per week.

Samples were collected during the weighing and addition tasks, with 12 short-term samples and 18 full-shift samples collected during the addition task and 4 full-shift samples during the weighing task. The short-term exposure data obtained were converted into 8 h TWA concentrations based on duration of the addition task and the number of batches per shift at each site. The HBCD levels are shown in Table 6.3.

Table 6.3. Inhalable HBCD concentrations from personal monitoring data collected from 4 EPS production plants in Europe for HBCD powder

Work task	Number of samples	Sampling duration in min, mean (range)	HBCD concentration (mg/m³)		
			Range	Median	90th percentile
Addition (short-term measurement)	12 (2 sites)	27 (13–56)	2.89–21.5	5.52	10.5
		480 (8 h TWA)	0.12–3.36	0.42	1.11
Addition (full-shift measurement)	18 (3 sites)	364 (275–504)	0.07–14.7	0.27	1.10
Weighing (full-shift measurement)	4 (2 sites)	259 (124–350)	4.35–12.1	6.19	10.5

From Table 6.3, the concentrations of HBCD used in the estimation of internal exposures are calculated based on the full-shift measurements obtained in the study, consistent with the occupational exposure estimation guidance by the European Chemicals Agency (ECHA, 2008b). These levels are considered representative of the addition and weighing tasks. The median (0.27 and 6.19 mg/m^3 for addition and weighing tasks, respectively) and 90th percentile values (1.1 and 10.5 mg/m^3 for addition and weighing tasks, respectively) in powder as shown in Table 6.3 are used as the typical and the reasonable worst-case levels for HBCD in air (C_{air}). The exposure duration is assumed to be 0.5 h/d for the addition task and 0.5 h/d for the weighing task. Using Equation 1 and the parameter assumption (Section 6.3.2), the typical and worst-case internal inhalation exposures from HBCD powder are 2.5 and 10.2 μg/kg bw/d (addition), and 58 and 98 μg/kg bw/d (weighing), respectively.

HBCD granules

Abbot (2001) reported a study conducted by the European Extruded Polystyrene Insulation Board Association on the measured airborne concentration of HBCD in the production of XPS resin from HBCD granules.

The study measured worker exposure to HBCD at 7 different sites in various European countries. Workers at these sites performed the same process for the purpose of exposure measurement. HBCD granules were delivered in 850 kg multiple use boxes with inliners. The main relevant tasks for exposure were the emptying of boxes and cleaning of the feed deck. The boxes were emptied into hoppers by a vacuum system and the solid stream was metered to the process using a closed system. Emptying the boxes using the vacuum system required about 15 minutes and was performed once daily. During this operation, dust particles were generated by attrition, and small leaks at the metering system occurred, which required manual cleaning. Feed deck cleaning was performed about once a week and required approximately 1 h.

A total of 43 samples were taken at fixed locations (representing typical operator positions) from 6 production sites in Europe. The studies provided both static and personal measured data. The samples were taken during emptying of the HBCD granules into a hopper by a vacuum system, and also during cleaning of the feed deck. Cellulose membrane filters were used for sampling, and then analysed specifically for bromine contents using an X-ray fluorescence technique. The results are shown in Table 6.4. For the samples with a HBCD concentration below the limit of detection (LOD), the value was taken to be 0.5 LOD.

Table 6.4. Summary of the results of the measurements in 6 plants using HBCD-granules for the production of XPS

Sample type	Number of samples	Sampling duration (h) mean (range)	Limit of detection (μg/m³)	Concentration (μg/m³) mean (range)
Static (feed deck)	7	11.9 (7.7–15.5)	4	7.43 (2–13)
Static (feed deck)	10	8	20	82.2 (10–220)
Static (feed deck)	6	6.21 (4.4–8)	20–34	137 (10–400)
Static (feed deck)	5	13.5 (5.3–23.9)	Not reported	560 (220–880)
Personal (routine work)	9	5.55 (3–7.3)	6–10	6.29 (3–15)
Personal (cleaning feed deck)	6	1	80	40

Generally, the concentrations of HBCD in the personal samples were lower than the static samples in the study. Using all the measured data and substituting 0.5 LOD for the non-detectable samples, the median and 90th percentile concentrations were 13 μg/m³ and 370 μg/m³, respectively. Approximately 50% of the samples had HBCD concentrations below the relevant LOD. As the median air concentration of 13 μg/m³ is lower than the LOD for a high proportion of the samples, the typical exposure level, for the task of adding and weighing HBCD granules, can be conservatively taken to be the highest LOD of 80 μg/m³. The 90th percentile value of 370 μg/m³ is assigned as the reasonable worst-case level.

The exposure duration is assumed to be 0.5 h/d for the addition task and 0.5 h/d for the weighing task. Using Equation 1, the typical and worst-case internal inhalation exposures from HBCD granules are 0.74 μg/kg bw/d and 3.44 μg/kg bw/d, respectively, each for the addition and weighing tasks.

Thomsen et al. (2007) investigated the exposure of workers to HBCD in an industrial plant in Norway producing EPS products, with the intent to compare the exposure with biomonitoring data for the workers. The particle sizes of the HBCD ranged from 20 nm to 2 mm. Concentrations of HBCD in airborne dust in the workroom were measured and workers with no known occupational exposure to HBCD were used as a reference group. Three people were working on each shift; 2 of these were performing different work tasks in the production process. These work tasks were referred to as reactor work (reactor room), including weighing and addition of HBCD to the PS granules in the reactor, and subsequent washing, centrifugation, sifting and transfer of the product to a silo container. The remaining worker on the shift conducted mixing procedures (mixer room) – a closed, automated process excluding potential contact with neat HBCD.

HBCD-containing products were produced in intermittent periods, 8 to 10 times a year, at the plant, with the production period lasting up to 14 d with 24 h operation divided into 3 8 h work shifts a day. Every production period was regularly followed by at least a 7 d period where products not containing HBCD were produced. Thirty airborne dust samples were collected during the production period: 24 samples from the reactor activities and 6 samples from the mixer procedures. The HBCD air concentrations in the workrooms are presented in Table 6.5. The internal exposure for this study was not estimated for comparison with the biomonitoring data, due to the high variability of the results.

Table 6.5. HBCD levels at the EPS industrial plant in Norway

Sample type	Number of samples	Mean sampling duration (h)	Concentration (μg/m^3)	Variability (μg/m^3)
Mixer room	6	8	0.5 (mean) 0.5 (median)	0.2–0.9
Reactor room	24	8	15.1 (mean) 2.7 (median)	1–150

Comparing the measured data for the addition and weighing tasks by Searl and Robertson (2005) and Abbot (2001), the median HBCD levels are up to 41 times greater for powder than for granules. The 90th percentile HBCD levels are up to 16 times greater for powder than for granules. Dermal exposure during addition and weighing.

No overseas measured data on dermal exposure were available.

6.5.5 Modelled exposures

Inhalation exposure during addition and weighing

The EASE operation description used is dry manipulation and the description of the solid used is non-aggregating, non-fibrous, with a particle size in the inhalable range. The full shift concentration is predicted by EASE to be 2–5 mg/m^3 with LEV and 5–50 mg/m^3 without LEV. These 2 ranges are used to represent the typical and the reasonable worst-case concentrations, respectively.

For HBCD powder, assuming 0.5 h/d duration for the addition task and 0.5 h/d duration for the weighing task and using Equation 1, the typical and worst-case internal inhalation exposures from HBCD powder are 18.6–46.4 μg/kg bw/d (with LEV) and 46.4–464 μg/kg bw/d (without LEV), respectively, each for the addition and weighing tasks.

For HBCD granules, the air concentration of HBCD is 10% of the EASE estimates for HBCD powder according to the assumption in Section 6.3.2. Assuming 0.5 h/d duration for the addition task and 0.5 h/d duration for the weighing task and using Equation 1, the typical and worst-case internal inhalation exposures from HBCD granules are 1.86–4.64 μg/kg bw/d (with LEV) and 4.64–46.4 μg/kg bw/d (without LEV), respectively, each for the addition and weighing tasks. These are the same concentration estimates as for the repackaging task (Section 6.5.2) indicating that it is reasonable to use the measured data for addition and weighing as surrogate measured data for the repackaging task.

Dermal exposure during addition and weighing

The EASE operation description used is direct handling and intermittent, non-dispersive use. The predicted external dermal dose is 0.1–1 mg/cm^2/d.

For HBCD powder, internal dermal exposures are 57–570 μg/kg bw/d, each for the addition and weighing tasks. For HBCD granules, internal dermal exposures are 29–290 μg/kg bw/d, each for the addition and weighing tasks. The internal doses following dermal exposure were calculated using the assumptions in Section 6.3.2 and Equation 2.

6.5.6 Comparison of measured data and modelled exposures

The internal inhalation exposures from the measured data and EASE modelling are shown in Table 6.6.

Table 6.6. Internal inhalation exposures for the addition and weighing tasks

	Task	I_{inh} (μg/kg bw/d) from measured data		I_{inh} (μg/kg bw/d) from EASE estimates	
		Typical	Worst-case	Typical	Worst-case
HBCD powder	Addition	2.5	10.2	18.6–46.4	46.4–464
	Weighing	58	98	18.6–46.4	46.4–464
HBCD	Addition	0.74	3.44	1.86–4.64	4.64–46.4

granules	Weighing	0.74	3.44	1.86–4.64	4.64–46.4

The EASE predictions can be seen to generally be overly conservative in comparison with measured data for the same scenarios. For the task of weighing HBCD powder, the typical exposure from the measured data is above the range given by EASE, while the measured worst-case exposure falls within the range given by EASE.

6.6 Exposure in the textile industry

HBCD is used in the textile industry as an additive to impart flame-retardant properties to textile products such as blinds and upholstery. Workers in the textile industry may be exposed to HBCD during formulation of flame-retardant coatings and during treatment of textiles with the flame-retardant formulations.

6.6.1 Formulation of flame-retardant coatings

In Australia, two companies provided information on using concentrated HBCD dispersions to formulate liquid coatings or fabric treatment formulations. Due to the HBCD being imported as a dispersion in water, the potential for inhalation exposure via dust is very low. The major route of exposure is therefore dermal contact with the HBCD dispersions.

One company uses an aqueous dispersion of HBCD with antimony trioxide to formulate liquid formulations for coating blinds. The concentration of HBCD in the dispersion is in the range of 30% to 60%. The final formulation has an HBCD concentration of less than 2%. Formulation is an open process that involves manual handling of the HBCD dispersion and automated mixing. The HBCD dispersion is measured using a dipper and is added to the basecoat in a drum on the weighing station. The mixture is then poured into an open mixing tank using a mechanical lifter, and mixed with a mechanical stirrer.

Three workers, working 6 h/d, are employed in the mixing room. The weighing of the 30% to 60% HBCD dispersion takes a maximum of 10 minutes per batch. The company estimates that 70% of the formulation batches are prepared with the HBCD concentrates. LEV is fitted in the mixing room and around the mixers. The mixing room workers were reported as wearing PPE, including safety goggles, respirators or dust masks and elbow-length gloves.

The other company uses an aqueous dispersion of HBCD with a surfactant to formulate a solution for the exhaustion treatment of polyester fabric. The HBCD in the dispersion is at concentration of 30% to 60%. The dispersion is decanted into mixing tanks and diluted with warm water to give a final HBCD concentration of 3% to 6%. The formulation is then transported to the dyehouse by trolley.

The 4 dyehouse operators involved in the manual decanting and transport of the HBCD dispersion were reported to wear PPE, including organic vapour respirators. The dyehouse was reported as a well-ventilated open space. Direct contact with the undiluted HBCD dispersion only occurs for approximately 5 minutes. A maximum of one to 2 batches are formulated per week.

6.6.2 Exhaustion treatment of polyester textiles

Exhaustion treatment of polyester fabric with a formulation containing 3% to 6% HBCD is carried out at one site in Australia in New South Wales. It is a semi-automated

process similar to exhaustion dyeing, in that the HBCD is exhausted onto the polyester fibres under high temperatures in a pressure vessel. The operators add the HBCD formulation into a side tank of the pressure-dyeing vessel. After heating the formulation and the fabric in a vessel, the vessel is cooled and the liquid content is drained. The fabric is further treated to remove any chemical residues from the exterior of the fabric, and then dried. As water and solvents are lost on drying, concentrations of flame-retardants in the coating layer will be higher than in the formulation. After treatment, the fabric contains between 10% and 20% HBCD by weight. Skin contact with HBCD is possible at the beginning of the cycle when the HBCD mixture is added to the side tank of the pressure vessel. Following this, the process is fully automated and there is no further contact with HBCD.

The manual handling step in the dyehouse takes approximately 5 minutes for each run. Less than 1% of fabric treatments, carried out by the company, are with the HBCD dispersion. Due to the automated nature of the process and the fact that HBCD is present in low concentrations (3%–6%) in liquid dispersions, occupational exposure is expected to be low.

6.6.3 Blinds coating

The use of HBCD formulations containing 2% HBCD to coat blinds is carried out at one site in Australia. This is a fully automated process. The formulated coating dispersion is pumped from a drum and applied to the polyester fabric using a doctor blade. The fabric then moves through a series of ovens (up to 150 °C) to dry the coated materials. This process is repeated to allow several coats of the fire-retardant formulation onto each side of the fabric.

At the blind coating site, the ovens are fully ducted to exhaust systems. The coating operators were reported to wear PPE, including overalls and eye protection. Due to HBCD being dispersed in water at low concentrations, the potential for inhalation exposure via dust is unlikely and the dermal exposure from limited manual handling is expected to be low. In addition, the automated nature of the process and the use of PPE will further reduce occupational exposure.

6.6.4 Measured data

No measured data are available in Australia or overseas for the use of HBCD dispersions during formulation of coatings and treatment of textiles.

6.6.5 Modelled exposures

Inhalation exposure during formulation

Inhalation exposure to HBCD during the formulation process is not estimated because HBCD is not expected to be present in air as a vapour or dust.

Dermal exposure during formulation

Dispersions containing 30% to 60% HBCD are formulated into coating solutions containing 2% HBCD for treating textiles or blinds. As the concentration of HBCD is highest in the original dispersion, the formulation process is likely to result in higher exposures to HBCD than the textile treatment processes. The formulation process is used to quantitatively estimate exposure in the textile industry.

The EASE operation description used is non-dispersive use and intermittent contact. The predicted external dermal dose is 0.1 to 1 mg/cm^2/d. Taking into account the maximum concentration of HBCD in the dispersions as 60%, and using the assumptions in Section 6.3.2 and Equation 2, the internal dermal exposures are 34 to 340 μg/kg bw/d.

6.7 Exposure of end users to HBCD-containing products

Several workers handle the semi-finished and end products containing HBCD in Australia. All of these products contain HBCD at low concentrations, with the HBCD either incorporated into a plastic matrix or fixed onto fibres. The most common products containing HBCD used in Australia are the plastic EPS foam products in the building industry and textile products treated with HBCD. From these products, 2 worker groups are expected to be exposed to HBCD: the building industry workers and upholstery workers.

Building industry workers installing polystyrene boards (EPS and XPS) will potentially be exposed to HBCD since the installation of the boards involves board cutting, either by hand or using hot wire cutting equipment. These processes may generate EPS or XPS particles. However, applicants have advised that the particles generated from cutting are unlikely to be small enough to be in the inhalable range. Additionally, the HBCD concentrations in these polystyrene boards are low (<5%). Therefore, minimal inhalation exposure of HBCD is expected for the building industry workers, and the inhalation exposure level is not estimated.

Since HBCD is incorporated into the plastic matrix, only low-level dermal exposure from possible leaching is likely during manual handling activities. However, this is considered to be negligible for building industry and upholstery workers.

6.7.1 Measured data

No measured data in Australia and overseas were provided for building industry and upholstery workers.

6.7.2 Modelled exposures

Inhalation exposure to upholstered products

Textiles treated with HBCD are used for the manufacture of a number of upholstered articles with a HBCD content in treated fabrics of 10% to 20% on a weight basis. For upholstery workers, the reasonable worst-case scenario is based on workers having contact with small debris of fibre containing HBCD in the dust generated during cutting and sewing fabrics and upholstering of furniture. A dust level of 50 μg/m^3 in the work area is selected as a reasonable point estimate to use in the absence of any measured data (enHealth, 2002) and half of the dust content is assumed to be the fabric debris containing 20% HBCD (f_{resp} = 0.2). Using these values, the assumptions in Section 6.3.2 and Equation 1, the internal inhalation exposure for an 8 h shift is 0.75 μg/kg bw/d.

Dermal exposure to end-use products

Dermal exposure to HBCD during from the end-use products is not estimated and is expected to be low.

6.8 Summary of occupational exposures

The internal inhalation (typical and worst-case values) and dermal exposures to HBCD in the occupational scenarios identified are presented in Table 6.7.

Table 6.7. Internal exposures of workers in HBCD industry scenarios

		Measured data (µg/kg bw/d)		Modelled exposure (µg/kg bw/d)	
Industry	**Activity**	**I_{inh}**	**I_{derm}**	**I_{inh}**	**I_{derm}**
Importation and repackaging	Repackaging (powder)	161 (typ) 216.4 (w-c)	None	74–186 (typ) 186–1860 (w-c)	57–570
	Repackaging (granules)	2.96 (typ) 13.76 (w-c)		7.4–18.6 (typ) 18.6–186 (w-c)	29–290
Polymer industry	Addition (powder)	2.5 (typ) 10.2 (w-c)	None	18.6–46.4 (typ) 46.4–464 (w-c)	57–570
	Weighing (powder)	58 (typ) 98 (w-c)		18.6–46.4 (typ) 46.4–464 (w-c)	57–570
	Addition (granules)	0.74 (typ) 3.44 (w-c)		1.86–4.64 (typ) 4.64–46.4 (w-c)	29–290
	Weighing (granules)	0.74 (typ) 3.44 (w-c)		1.86–4.64 (typ) 4.64–46.4 (w-c)	29–290
Textile industry	Formulation (liquid)	None	None	Negligible	34–340
End uses	Upholstering	None	None	0.75	Negligible
	Building			Negligible	Negligible

typ – typical; w-c – reasonable worst-case.

In Table 6.7, the internal exposures of workers in the repackaging tasks reasonably used the measured concentrations of airborne HBCD from the polymer industry as a surrogate based on the similarity of tasks for repackaging (Section 6.4) and weighing and addition in the polymer industry (Section 6.5). The estimated exposure values utilised the assumptions in Section 6.3.2 and Equation 1, and task duration of 2 h/d.

Inhalation and dermal internal exposures activities involving HBCD powders are higher than the tasks associated for HBCD granules. The reasonable worst-case inhalation exposures calculated by EASE are for the case where when LEV is used, and are significantly lower that the worst-case estimates in the absence of LEV. The reasonable worst-case dermal exposures do not take into account PPE, and normal work clothing would be expected to considerably reduce exposure.

The internal inhalation exposures derived from measured data are generally within the range of the estimates that used EASE modelling. The upper range of the modelled reasonable worst-case inhalation exposures is up to 45 times the estimated inhalation

exposures from measured data. The results demonstrate the conservative nature of the EASE model.

7. Environmental exposure

7.1 Introduction

The environment is potentially exposed during all stages of a chemical's life cycle. Each release is considered separately during:

- processing/formulation (site-specific, point source releases)
- use (point or diffuse release, depending on use pattern)
- disposal.

Regardless of the environmental media being assessed for exposure, within the exposure assessment there are 3 main steps, the ultimate one being to derive predicted environmental concentrations (PECs) in relevant environmental media. These steps are:

1. release estimation
2. consideration of environmental fate and partitioning behaviour (distribution)
3. PEC derivation.

When assessing environmental exposure of existing chemicals, previous releases can also be considered through the use of monitoring data where available. Monitoring data are discussed at the end of this section. These data will help establish background concentrations for use in the risk assessment or, if relevant, be used to add certainty to the PECs. For this national assessment approach, calculations are performed to represent realistic worst case situations.

Importation data are presented in Chapter 4 and show total quantity of HBCD (tonnes) over the period 2003 to 2010. The total volumes imported during the period 2007–08, 2008–09 and 2009–10 (86, 55 and 60 tonnes respectively) have fallen significantly. However, Chapter 4 also notes that, prior to this period, industry had reported imports averaging to around 80 tonnes (2005–08). Release estimates undertaken in this section have been performed on the historical values as use of HBCD in articles from pre-2008 import levels are still likely to remain in service and are yet to enter the waste stream.

7.2 Quantifying release to the environment

Release into the environment may occur during manufacture, importation, repackaging, processing and use.

7.2.1 Local release during importation and processing

As HBCD is not produced in Australia, potential release from manufacture site is not considered in this assessment. Local releases are likely to result during incorporation of imported pure and liquid dispersions of HBCD into the intermediate plastics, and from processing of these plastics into end-use products.

Raw material handling

Initially some emissions will be to the atmosphere, but ultimately all particulates will be removed or settle and losses will be to solid waste or wastewater as a result of wash-down. Emissions to wastewater may also occur in the event of paper sacks being recycled at a different location. The OECD emission scenario document (ESD) (OECD, 2004a and 2004b) reports release estimations for powders of 2 particle sizes, namely <40 μm and >40 μm. The smaller size only will be used in this assessment.

For powders of particle size <40 μm, the loss factors for a realistic worst case scenario are:

$F_{handling, water}$ = (0.1%+ 0.5%) = 0.6% to solid waste/water

$F_{handling, air}$ = 0%

$F_{handling, waste}$ = 1.0% to solid waste as residue in bags

F = the fraction of release.

When the initial assessment for HBCD was undertaken in 2006, flame-retarded EPS resin containing HBCD was produced at one work site in Australia. Releases from handling of the raw material, based on an annual volume of 50 tonnes HBCD in its powder form, will result in an estimated release to water of 250 kg with a further 550 kg being released as solid waste (50 kg through handling and 500 kg as residues in bags). Advice received in 2011 is that this is no longer done. However since this process was being used in Australia till 2010 and since HBCD is a persistent chemical, the amount of HBCD released to the environment will persist for long periods in the environment, calculations relating to this activity have been retained. It is also possible that these activities will be recommenced in Australia unless regulated.

Compounding (formulation)

Again, initially some emissions will be to the atmosphere, but ultimately all particulates will be removed or settle, and vapours will condense to some extent, resulting in losses to both solid waste and wastewater (via aqueous washing). It will be assumed here that all particulate losses will eventually be to wastewater and that volatilisation loss will condense to some extent and eventually be released 50% to air and 50% to wastewater. These assumptions are included in the emission factors given below.

The ESD provides release estimates for flame retardants falling into 3 categories of low, medium and high volatility. In this regard, the ESD does not define volatility groups based on flame retardant vapour pressure characteristics. The basis for assigning chemicals to the groups is not provided. One of the plasticisers, di(2-ethylhexyl) adipate (DEHA) that has a vapour pressure very similar to HBCD (6.27×10^{-7} hPa) has been assigned to the high volatility group in the ESD. Therefore, HBCD will be assumed to fall in the high volatility group and only these emission estimations are made here:

For powders of particle size ≤40 μm	High volatility group	$F_{compounding, water}$ = 0.075% $F_{compounding, air}$ = 0.025%

With only one site having undertaken production of flame-retarded EPS resin containing HBCD, release to water and air at this site over 1 year (based on 50 tonnes per annum) is estimated to be 37.5 kg to water and 12.5 kg to air.

Conversion (processing)

Releases described by the ESD for organic flame retardants are considered here, as this is more relevant to HBCD. It is unclear how many sites undertake processing operations using flame-retarded EPS in Australia.

These losses will initially be to air at elevated temperatures. However, subsequent condensation could result in losses to liquid waste. On the basis of volatile losses the loss factors for realistic worst-case conditions are as follows. Factors apply to the quantities used at the plastics processing site. As a realistic worst case, it could be assumed that 50% is lost to wastewater and 50% to the atmosphere. This assumption has been used in the emission factors reported below, all for the high volatility group of flame retardants:

Open processes	solid articles	$F_{conversion, water}$ = 0.125%
		$F_{conversion, air}$ = 0.125%
Open processes	foamed articles	$F_{conversion, water}$ = 0.25%
		$F_{conversion, air}$ = 0.25%
Partially open process		$F_{conversion, water}$ = 0.075%
		$F_{conversion, air}$ = 0.075%
Closed processes		$F_{conversion, water}$ = 0.025%
		$F_{conversion, air}$ = 0.025%

For processing at significantly in excess of 200 °C, loss factors should be increased by a factor of 10. Many conversion processes operate at around 200 °C, with perhaps only a relatively small proportion of production operating at temperatures significantly higher than this. However, that proportion is not negligible and will include virtually all the engineering plastics such as the polycarbonates, polyamides and PET. Such higher temperature operations could include the oven post-cure of polyamide (open), the extrusion of polyester film (partially open) or various materials in injection moulding (closed). An upper limit for all these might reasonably be 300 °C. The ESD further notes that some amorphous polymers such as polystyrene are processed at temperatures up to 250 °C.

To accommodate the likelihood of such higher temperature operations, it will be assumed that 10% of the HBCD is processed using conversion processes at temperatures in excess of 200 °C.

The ESD notes that for smaller sites (<750 tonnes of plastic per year) loss factors should be increased by a factor of 10. Again, the extent of such operations in Australia is unclear. For this assessment, it will be assumed that 50% of the processed HBCD is performed in plants processing <750 tonnes of plastic per year.

Conversion operations using EPS are assumed to be predominantly in closed systems. Imported EPS containing HBCD has averaged 33 tonnes over the 3 years between 2005 and 2008. Combined with the 50 tonnes processed into EPS in Australia, an annual total of 83 tonnes in EPS resin will be available for conversion. Using the above assumptions relating to processing temperatures and quantities of plastic produced, the estimated releases through conversion processes are shown in Table 7.1.

Table 7.1. Annual releases (kg per annum) from conversion processes of EPS resin containing HBCD in Australia

	Air	Water	Solid waste
Conversion, <200 °C, <750 t plastic p.a.	93	93	–
Conversion, <200 °C, >750 t plastic p.a.	9.3	9.3	–
Conversion, >200 °C, <750 t plastic p.a.[1]	20.8	20.8	–
Conversion, >200 °C, >750 t plastic p.a.	10.4	10.4	–

1) The annual release is estimated to be 0.67 kg based on the release factor of 0.025%. Two factors of 10 have to be added to this release estimate due to high temperatures (>200 °C) and small operations (<750 tonnes per annum). These factors are considered additive (that is, 0.67 x 10).

Liquid dispersions of HBCD

Two forms of liquid dispersions of HBCD are imported into Australia. One contains HBCD at 15% to 30% in a liquid dispersion with antimony trioxide. This product is used in polymer coatings applied to polyester and polyester/cotton blend fabrics used to manufacture vertical, holland and roman blinds. Based on information provided in Chapter 4, a maximum amount of 3.913 tonnes of HBCD is estimated for these uses.

The other product contains HBCD at 30% to 60% HBCD in a liquid dispersion with an anionic surfactant and is used in the manufacture of automotive and technical textiles, where it is added to the dye bath in an exhaust dyeing process. End uses of these textiles include automotive and public seating products and protective wear garments for military and industrial purposes. Based on information provided in Chapter 4, a maximum amount of 1.83 tonnes HBCD is estimated for these uses.

Releases from these 2 distinct processes are considered separately and are based on the OECD emission scenario document on the textile finishing industry (OECD, 2004b).

Blinds: During the coating of blinds with HBCD dispersion, approximately 99% of the coating liquors remain on the textile (OECD, 2004b). Hence, for a realistic worst-case estimation $F_{residual\ liquor} = 0.01$ would be the default value. The degree of fixation in this case is $F_{fixation} = 1$.

Because this assessment is only focusing on release of the flame retardant, full release formulation details provided in the ESD are not considered necessary. The fixation rate of 1 means that the release to water will be a function of the fraction remaining in the residual liquor – that is, 1%.

Based on 13 tonnes per annum being used in textile coating, 1% release to water will result in a predicted annual release of 130 kg HBCD.

In the ESD, emissions to air are calculated based on several factors such as liquor concentrations, liquor pick-up, textile substrates and finishing temperature. Given the generic nature of this assessment and that many variations are possible, for this assessment, release estimation to air has been simplified and is based on the A-Tables (emission factors) of the European Union Technical Guidance Document (TGD) (EC, 2003) from IC-11 (polymer industry) life cycle phase "polymer use". Release fractions to air (F_{air}) for substances with boiling points >300 ^{o}C and soil (F_{soil}) are set at 0.0005 and 0.0001 respectively.

Based on annual usage in textiles of 13 tonnes per annum, annual releases to air and soil are calculated to be 6.5 and 1.3 kg respectively.

Automotive and technical textiles: When used in the manufacture of automotive and technical textiles, the liquid dispersion of HBCD is added to the dye bath in an exhaust dying process. As explained in the ESD, exhaust dyeing is always carried out in a discontinuous way and involves applying of a dyestuff in solution or suspension at a specific liquor ratio, which determines the depth of the colour obtained. At the end of the dyeing operation the spent dye-bath liquor is drained off. The post-dyeing stage consists of washing with water to remove unfixed amounts of dyestuff from the textile substrate. In some cases, soaping and special after treatment steps are necessary.

Through these processes, release to water can occur at the following discharge stages:

- residual baths from exhaust processes (especially exhausted dye baths)
- washing and rinsing steps which follow the dyeing process
- after-treatment baths (soaping or reductive after-treatment subsequent to dyeing)
- residual padding liquors, residual printing pastes and residual coating pastes
- cleaning the machines.

Formulators have estimated that the exhaustion of the dye on to the fabric is in the order of 95% to 99%. However, no data were provided to support this estimate.

Considering all possible routes of release to discharge water described above, a more conservative estimation will be used. The ESD provides a fixation default value for auxiliaries (a term that includes flame retardants) intended to fix to textiles during an exhaust dyeing process of 0.8. For this assessment, this will be taken to be the total amount remaining on the textiles after all possible discharge stages – that is, 20% of the initially applied chemical will be estimated to be released with discharge water during the dyeing and subsequent operations, and machine cleaning.

Based on a maximum annual use of 3000 kg HBCD in this industry, annual release to water is therefore estimated to be 600 kg.

Estimation to air has been simplified and is based on the A-Tables (emission factors) of the European Union Technical Guidance Document (TGD) (EC, 2003) from IC-13 (textile processing industry). For processing operations, this document provides an emission factor to air for flame retardants (use category 22) where the solubility is <100 mg/L and the vapour pressure <100 Pa of 0.05.

Based on annual usage in textiles of 3000 kg per annum, annual release to air is calculated to be 150 kg.

The annual local releases from processing and conversion of HBCD in the plastics and textile industries in Australia are summarised in Table 7.2:

Table 7.2. Summary of releases from processing of HBCD into plastics and textiles

Stage of operation	Release (kg per annum) Air	Water	Soil/solid waste[1]
Plastics industry – processing (1 site only – technical grade granule)			
Handling	–	250	550
Compounding	12.5	37.5	–
Plastics industry – processing, ESP resin conversion			
Conversion, <200 °C, <750 t plastic p.a.	93	93	–
Conversion, <200 °C, >750 t plastic p.a.	9.3	9.3	–
Conversion, >200 °C, <750 t plastic p.a.	20.8	20.8	–
Conversion, >200 °C, >750 t plastic p.a.	10.4	10.4	–
Textile industry – processing, liquid dispersion, blinds (1 site only)	6.5	130	1.3
Textiles industry – processing, liquid dispersion, automotive and technical textiles	150	600	–
Total	302.5	1151	551.3

1) It is assumed this is disposed of to landfill.

7.2.2 Release during service life of long-life articles

To account for emissions during service life of the long-life articles containing HBCD, the HBCD products formulated in Australia as well as HBCD imported in ready-made articles must be considered.

Plastics

Loss of additives over the service life of polymers can be considered to occur through volatilisation to air and leaching to water. This may occur, for example, through "blooming", where the chemical moves to the surface, from which it may volatilise, flake off or be washed off. The use of polymers is widely dispersed and so the service life emissions are considered on a regional level rather than a local level. Some emission factors may include particulates caused by abrasion/degradation of the article. Ultimately, all particulates will be removed or settle and losses will be to soil or water as a result of wash-down. Therefore, as a realistic worst case, it could be assumed that all this loss will eventually be released to water. Some of the emission factors also depend on the service life of the article.

The yearly emissions of additives during the service life of polymers can be estimated from the following equations (OECD, 2004a):

$$RELEASEtot\_STST_{polymer,\,air} = F_{service\ life,\ air} \times Q_{tot\ polymer} \times 1000$$

$$RELEASEtot\_STST_{polymer,\,water} = F_{service\ life,\ water} \times Q_{tot\ polymer} \times 1000$$

where:

$RELEASEtot\_STST_{polymer,\,air}$	annual total release of the substance to air over the service life of the product at steady	kg p.a.

	state	
$RELEASEtot\_STST_{polymer, water}$	annual total release of the substance to air over the service life of the product at steady state	kg p.a.
$F_{service\ life,\ air}$	emission factor to air over service life of the polymer product	%
$F_{service\ life,\ water}$	emission factor to water over service life of the polymer product	%
$Q_{tot\ polymer}$	annual total input of the substance into the polymer product	tonnes p.a.

The ESD provides the following emission factors:

Indoor service, leaching to liquid waste	Fservice life, water = 0.05% over lifetime
Indoor service, volatility to atmosphere	Fservice life, air = 0.05% over lifetime
Outdoor service, leaching to environment	Fservice life, water = 0.16% x Tservice (where Tservice = service life of product in years)
Outdoor service, volatility to atmosphere	Fservice life, air = 0.05% over lifetime

The majority of HBCD flame-retarded EPS resin (63%), both manufactured up to 2010 in Australia and imported as unexpanded EPS beads, is used in the moulding of insulation panels, sheets and blocks for use in the construction of industrial and residential buildings. This will account for up to 55 tonnes HBCD per annum. These products are used in various ways, including as exterior sheathing and in subfloor systems, exterior walls, ceilings and upside-down roofs (protected membrane roofs). However, they are not likely to be directly exposed to the environment and are therefore deemed to be in indoor service for release estimation. Release to the environment over the product's life is expected to be very small, as these panels are not likely to be exposed.

Emission experiments have been conducted to measure the loss of HBCD from foamed polystyrene (Klatt, 2004). Air was blown at a rate of 15 L/h through a tube of EPS–XPS, containing 1.1 and 2.0% HBCD with an inner surface area of 0.15 m^2 (4 cm diameter and length of 120 cm). The emitted HBCD was adsorbed on PUR-foams. After the sampling period the PUR-foams were extracted and HBCD was determined by LC/MS with a detection limit of about 5 ng HBCD per sample. The maximum amount found after 5 d was about 10 ng HBCD. Taking into account the surface of the tube, the emission can be calculated to be 70 ng/m^2 per 5 d, corresponding to 5 $\mu g/m^2$ per year.

In order to consider this result quantitatively, an estimate of the surface area of insulation panels containing HBCD used in Australia each year is required. The dimensions of the panels are given as 600 mm x 300 mm x 2400 mm. This gives a surface area of 1 panel of approximately 4.7 m^2 (total of 6 faces). Most EPS imported into Australia are SL grade 13.5 kg/m^3 (0.0135 g/cm^3).

This means each board will weigh between 5.8 kg. HBCD is present in the insulation boards at <5% by weight. Assuming 1% as a realistic worst case, a total of 5500 tonnes of EPS will be manufactured/imported per annum, or the equivalent of 950000 boards, depending on the density. This results in a maximum surface area of the boards of around 4.45 million m^2. With a release rate of 5 $\mu g/m^2$ per year, this results in an annual maximum release to air of 0.022 kg, which is substantially lower than those predicted using the ESD default release fractions.

Given the use pattern in insulation panels, the following release estimates are probably an overestimate. However, when used on-site, moulded EPS blocks can be shaped at the building site, often with a handsaw. This will result in particle release to soil (then available for wash-off in storm water) or the atmosphere. There are no data on the actual amount of release through this exposure route, so they will be assumed to be included in the above release estimates.

The ESD suggests a service life of >10 years for plastics used in building and construction. The service life will be linked to the life of buildings they are used in. A service life of 20 years will be used for release estimates.

A second major use of the EPS materials containing HBCD (17%) is in the manufacture of miscellaneous industrial packaging, including packaging used for whitegoods. This will account for up to 14.8 tonnes of HBCD per annum. The ESD suggests a service life of 2 years for packaging. It will be assumed for these release estimates that the packaging is in indoor service.

A third major use of EPS materials containing HBCD is for use as beanbag fill (20%). This will account for up to 17.4 tonnes per annum. While no standard service life is available, it will be assumed all is in indoor service and will be replaced annually.

Based on this, the regional releases to air and water through the diffuse service life of plastic products containing HBCD are summarised in Table 7.3.

Table 7.3. Estimated regional releases to air and water of HBCD through service life of plastic articles

Function	Quantity p.a. (t)	Air (kg/year)	Water (kg/year)
Insulation panels	55	1.4	1.4
Packaging	14.8	3.7	3.7
Beanbag fill	17.4	8.7	8.7

Textiles

HBCD is used in the textile industry as a flame retardant in polymer coatings applied to fabrics in the manufacturing of vertical, holland and roman blinds, or in the manufacture of automotive and technical textiles for use in automotive and public seating products, and protective garments for military and industrial purposes.

The ESD (OECD, 2004b) indicates a service life for sun blinds of 8 to 15 years. While it does not indicate a service life for textiles used in seating products, these should be relatively long-lived. The ESD for the plastic industry (OECD, 2004a) suggests a service life of 10 to 20 years for plastics used in the transport and automotive industry. The other use pattern – protective garments for military and industrial purposes – has a suggested service life of 2 to 5 years in the ESD ("other clothes and bed linen").

There are insufficient data to determine the split in usage of HBCD between seating and protective clothing uses. However, an arbitrary split of 50:50 will be used for this assessment. Therefore, the following annual amounts and service lives as presented in Table 7.4 will be used for release estimation for HBCD used in the textile industry.

Table 7.4. Quantity of HBCD (annual) in various textile articles and their indicative service life

Use	Annual quantity (kg)	Service life of articles
Sun blinds	13 000	10 years
Automotive and public seating	1500	10 years
Protective garments for military ans industrial use	1500	2 years

Data on recycling of textiles (reuse as garments, reuse in spinning mills or non-woven manufacturing, and the reuse of man-made fibres in polymer processing) are not available. This item is therefore not taken into account in the ESD.

The ESD provides calculations for estimating release during the service life. These calculations essentially correspond to those described above for plastics, so the above formulae will be used. Default emission rates over the service life will be used as per those provided for plastics. It will be assumed that all uses will be indoors.

Based on this, the following releases to air and water through the diffuse service life of textiles containing HBCD are summarized in Table 7.5.

Table 7.5. Estimated releases to air and water of HBCD through service life of textile articles

Function	Quantity p.a. (t)	Air (kg/year)	Water (kg/year)
Sun blinds	13	0.65	0.65
Seating	1.5	0.08	0.08
Protective clothing	1.5	0.4	0.4

7.2.3 Delayed release from waste disposal

Losses from polymers at disposal can be considered as widely dispersed and so are considered on a regional level rather than a local level. The current waste streams do not necessarily reflect future situations that may occur through such avenues with better recycling and disposal regimes; however, at this stage landfill represents the most likely disposal situation for HBCD contained in articles.

For incineration, organic substances should be destroyed, so $F_{disposal, air} = F_{disposal, water} =$ 0% for incineration. There may be residues of inorganic materials left in the ash, which will be disposed of as solid waste (OECD, 2004a). Currently no incineration occurs in Australia.

The operation and construction of landfills varies throughout Australia and representative data on a national level are not available. After the technical lifetime of a landfill, a low but long-lasting flow of non-degraded substances into the environment will take place.

The main routes of emissions of substances from landfills are identified as leaching with water, transport with landfill gas and diffusion to the atmosphere, with the most important route depending on the properties of the substance. In the case of HBCD, transport with landfill gas and diffusion to the atmosphere are unlikely to be relevant due to its low vapour pressure. However, it could be found in the particulate phase following abrasion from articles and release to the atmosphere with dust.

Emissions of organic chemicals will be influenced by the degree of degradation in the landfill. To this end, information on the anaerobic degradability is needed, and data available, as discussed separately in this section, indicate that the rate of aerobic and anaerobic degradation is likely to be very slow.

In general, measured long-term emission data of sufficient analytical quality and knowledge of chemical composition of the landfilled waste are lacking.

If it is assumed that, on balance, the amount of product containing HBCD produced/imported each year replaces that disposed of each year then the amount of HBCD disposed of in plastic/textile products in Australia could be around 65 tonnes per year, based on the calculations discussed above and noting that additional volumes may be imported in articles that are unknown and, therefore, unaccounted for in the above calculations.

When HBCD in polymers is disposed of to landfill, it could leach out and into groundwater or volatilise to the atmosphere. Abrasion of discarded polymers may release particles containing HBCD within the landfill and this may provide a transport mechanism for entry into leachate water. However, the importance of these processes can simply not be assessed with currently available data.

Nonetheless, there is evidence that HBCD can migrate out of landfills. As part of a wider study, de Boer et al. (2002) undertook a sewage sludge and landfill study. STP and landfill samples were taken in The Netherlands, UK and Ireland. Dutch landfill samples were taken at 9 locations. At 2 locations both sludge and leachate water were sampled. The sampled leachate water was not identical to the water that finally reaches the open environment. The Dutch landfills were sampled after a period of several weeks of dry weather, which means that the concentrations found may be higher than those in the average situation with more rainfall. The leachates were filtered and the residue was dried at 50 °C and analysed. Three landfill leachate samples from the UK and 3 Irish landfill samples were analysed. All leachate water samples were split into particulate and dissolved parts for analysis.

While no HBCD was found in landfill leachates (either dissolved or particulate) from the UK and Irish sites, the Dutch samples showed HBCD concentrations in particulates in leachate water between <29 and 660 μg/kg on a dry weight basis, with 2 extreme values of 22 000 and 68 000 μg/kg dw. The median value for the leachate water, ignoring the 2 very high values was 178 μg/kg dw. Two sludge samples showed HBCD concentrations that were much lower than in the corresponding leachate water (2.1 and <0.3 μg/kg, respectively). Both α- and γ-HBCD were present in Dutch landfill samples. Normally γ-HBCD was somewhat higher in concentration, but α-HBCD was also present in substantial amounts in some cases.

7.2.4 Summary of releases

Table 7.6 below provides a summary of estimated releases of HBCD to the Australian environment through processing and end use of products containing this substance.

Table 7.6. Summary of releases from processing of HBCD into plastics, rubber and textiles

Life cycle stage	Release (kg per annum)		
	Air	Water	Solid waste[1]
Processing (local release)			
Plastics industry – processing (1 site only – technical grade granule)			
Handling	–	250	550
Compounding	12.5	37.5	–
Plastics industry – processing, EPS resin conversion			
Conversion, <200 °C, <750 t plastic p.a.	93	93	–
Conversion, <200 °C, >750 t plastic p.a.	9.3	9.3	–
Conversion, >200 °C, <750 t plastic p.a.	20.8	20.8	–
Conversion, >200 °C, >750 t plastic p.a.	10.4	10.4	–
Textile industry – processing, liquid dispersion, blinds (1 site only)	6.5	130	1.3
Textiles industry – processing, liquid dispersion, automotive and technical textiles	150	600	–
Total	302	1151	551
Service life of long life articles (diffuse release) regional			
Plastics industry			–
Insulation panels	1.4	1.4	–
Packaging	3.7	3.7	–
Beanbag fill	8.7	8.7	
Textiles industry			
Sun blinds	0.65	0.65	–
Seating	0.08	0.08	–
Protective clothing	0.4	0.4	–
Total for emissions during service life	14.9	14.9	–
Disposal			
Total emission from disposal	–	–	~85 000[2]

[1]It is assumed this is disposed of to landfill.

[2]Assumes importation and disposal/loss volumes are at equilibrium. Based on approximate annual importation of 87 tonnes, with around 2 tonnes per annum lost through processing and service life.

7.3 Environmental fate and partitioning behaviour

7.3.1 Data for exposure prediction/modelling

Before exposure concentrations are estimated, the environmental fate and pathways are examined by considering the physico-chemical properties and environmental fate properties such as persistence in different environmental media and partitioning behaviour.

Whenever possible, the evaluation of environmental exposure is done quantitatively, using appropriate mathematical models (e.g. a generic fugacity model). Otherwise a qualitative analysis should be undertaken, using a conceptual fate model (OECD, 1999).

7.3.2 Physical and chemical properties

The physical and chemical properties of HBCD have been discussed in Section 3. Physico-chemical parameters used for modelling were vapour pressure of 6.27 x 10^{-5} Pa, LogKow of 5.62, melting point of 180 ^{o}C (calculated with EPIWin) and water solubility of 0.0034 mg/L.

These factors indicate that, where released to the environment, HBCD will partition strongly to organic carbon and be unlikely to volatilise to the atmosphere. However, the Henry's Law Constant (calculated as 1.167 x 10^{-4} atm.m^3/mol) indicates that, where available in the water column, HBCD may volatilise to the surrounding atmosphere. Where HBCD is found in water and the atmosphere, it is most likely to be sorbed to particulate matter.

7.3.3 Persistence and partitioning

The following fate properties are important for determining the overall partitioning and fate behaviour of a substance in the environment:

- abiotic degradation (hydrolysis, photolysis)
- biodegradation
- partitioning behaviour (e.g. bioaccumulation, soil adsorption/desorption)
- persistence in environmental media.

1) Abiotic degradation

i) Photolysis

No photolysis data have been provided for HBCD, and none are described in the IUCLID. Therefore, it is not assumed to be a degradation pathway.

ii) Atmospheric photooxidation

The low vapour pressure indicates that the atmosphere is unlikely to be a significant compartment for HBCD to partition to unless it is sorbed to airborne particulate matter. The breakdown of HBCD through reaction with hydroxyl radicals in the atmosphere is likely to be very slow.

A rate constant for reaction of HBCD with OH radicals in the atmosphere was calculated using the AOP program [AOPWIN Program (Atmospheric Oxidation Program for Microsoft Windows 3.1) Version 1.91, Syracuse Research Corp. 1988–97].

The structure of 1,2,5,6,9,10-HBCD (CAS 3194-55-6) was entered into the program with the following SMILES notation:

BrC(C(Br)CCC(Br)C(Br)CCC(Br)C(Br)C1)C1.

The rate constant k_{OH} of the active substance was estimated based on the chemical structure:

k_{OH} = 5.015 x 10^{-12} cm^3/molecule/s

The half-life is calculated using the following equation:

$t_{½}$ = ln2/k' = ln2/k_{OH} x [OH radicals]

Using the half-life equation given above, and the diurnally and seasonally averaged concentration of tropospheric hydroxyl radicals of 1.5 x 10^6/cm^3, the half-life for the degradation of HBCD by hydroxyl radicals (12:12 h light:dark) was calculated to be 51 h.

iii) Stability in water

Hydrolysis

No data for hydrolysis of HBCD are available. The very low water solubility of the substance makes such testing difficult to perform, and it is expected to be resistant to hydrolysis under environmental conditions.

QSAR modelling using HYDROWIN v 1.67 indicates very long residence time in water with hydrolytic half-lives >1 X 10^{10} years at pH 7 and 8. Such results need to be used cautiously but do provide further support for a conclusion that hydrolysis is unlikely to represent a significant removal route for HBCD in the environment.

Volatilisation

HBCD has a Henry's Law Constant of 1.167 x 0^{-4} atm.m^3/mol (calculated from VP/Wsol). Based on the scale of Mensink et al. (1995), this substance has the potential to volatilise from water bodies (substances with a Henry's Law Constant >7.34 x 10^{-4} atm.m^3/mol are classified as highly volatile from water). The importance of this mechanism in reality should be mitigated by the strong sorption of HBCD to suspended material where it is more likely to end up in the sediments.

2) Biodegradation

To assist with this assessment, several laboratory studies addressing biodegradation of HBCD in a range of aerobic and anaerobic test systems have been provided. Often these tests were in the same report but have been split into the individual components in this assessment. Table 7.7 provides an overview of these studies. In the following analysis, results have been supplemented where possible from other published reports. Results are presented in summary form here. A more comprehensive description of the studies is provided in Appendix 4.

Generally, the studies reported by Davis et al. (2004) are considered better quality for characterising the biodegradation of HBCD. The test concentrations were significantly higher than those in Davis et al. (2003a; 2003b). These high concentrations in control tests were not toxic to microorganisms in the test systems, and allowed sufficiently high levels to enable quantification of the individual isomers and follow their degradation and metabolite formation. Additionally, ^{14}C-HBCD was used in the studies, thereby allowing the chemical to be "followed" in better detail throughout the tests. While the results reported in Davis et al. (2003a; 2003b) consider separate isomers, the level tested was too low to enable quantification of the α and β isomers. Hence the results are considered to be in terms of total HBCD for this assessment.

Briefly, HBCD is not ready biodegradable indicating it will persist under aerobic conditions. While degrees of degradation were found, the actual results as described below are subject to considerable interpretation. Degradation was not adequately demonstrated in soil. The more reliable studies from sediment tests indicate a half-life of 117 d in aerobic sediment and 75.3 d in anaerobic sediment, but these results need to be treated with caution – for example, true aerobicity was not established in the aerobic test and, in the anaerobic sediment test, the half-life does not represent mineralisation, so persistent compounds are still present. It is concluded that HBCD will be persistent in the environment and this is supported by monitoring data presented later in this report.

Table 7.7. Summary of laboratory test reports of HBCD biodegradation

Biodegradation	Test concentration / comments	Results[e]	References
Aerobic biodegradation			
Ready biodegradation		Not ready biodegradable	Schaefer and Haberlein, 1996
Aerobic sludge (inherent biodegradation)	3.6 mg/L[a]	~22% degradation after 56 d	Davis et al., 2004
Aerobic soil	0.025 mg/kg[a]	DT50 = 63 d	Davis et al., 2003a, 2005
	3.04 mg/kg[a]	Little degradation after 112 d	Davis et al., 2004
Aerobic water/sediment	0.034–0.06 mg/kg [b, c]	DT50 = 11–32 d	Davis et al., 2003b
	4.67 mg/kg [c, d]	DT50 = 117 d	Davis et al., 2004
Anaerobic biodegradation			
Anaerobic sludge (inherent biodegradation)	4.2 mg/L. Anaerobic conditions demonstrated (redox)	~87% degradation after 60 d	Davis et al., 2004
Anaerobic soil	0.025 mg/kg [e]	DT50 = 6.5 d	Davis et al., 2003a, 2005
Anaerobic water/sediment	0.063-0.089 mg/kg. Low recovery [c, f]	DT50 = 1.1–1.5 d	Davis et al., 2003b
	4.31 mg/kg [c, f]	DT50 = 75.3 d	Davis et al., 2004

a) Aerobic conditions demonstrated based on headspace oxygen concentration.
b) Water aerobic conditions demonstrated through redox potential, 1 system marginal.
c) Sediment aerobicity not measured in test system.
d) Fully aerobic conditions in water unclear, that is redox potential <200 mV.
e) Anaerobic conditions present based on resazurin dye, but not based on redox potential.
f) Fully anaerobic conditions in water unclear, that is redox potential often >50 mV.
g) These results should not be taken at face value and are discussed in context below.

In summary, where paired testing was performed – that is, the same laboratory study considered aerobic and anaerobic degradation in similar test systems – anaerobic degradation was faster than aerobic degradation. Additionally, some concentration dependence could be found. For example, tests with high concentrations (e.g. 3–4 mg HBCD/kg sediment) showed slower degradation than those with low test concentrations (e.g. 0.025 mg HBCD/kg sediment). Degradation was fast in anaerobic sludge. A further literature paper described below but not included in the above table supports this conclusion. Gerecke et al. (2006) demonstrated very rapid loss of all 3 HBCD isomers tested at low concentrations (2.5 ppb) in sludge and total HBCD degraded with a half-life <1 d.

i) Aerobic

Ready biodegradation

HBCD is not ready biodegradable based on a standard ready biodegradation study (Schaefer and Haberlein, 1996).

Inherent biodegradation

The transformation of ^{14}C-HBCD was determined under aerobic conditions using activated sludge taken from a municipal wastewater treatment plant (Davis et al., 2004). In the aerobic sludge, concentrations of total HBCD decreased from 99.1% applied radioactivity (AR) to 77.7% AR after 56 d (decreases of 6.8%, 27.3% and 22.3% for α-, β- and γ-HBCD respectively). Formation of ^{14}C-products was negligible and remained <2% throughout the study. HBCD was shown to not be inhibitory to the digester sludge at the tested rate. HBCD cannot be considered inherently biodegradable based on this test.

Soil

The transformation of HBCD was determined in soils based on OECD TG 307 and GLP (Davis et al., 2003a; 2005). The studies were conducted in laboratory batch microcosms prepared with a surface soil (top 15 cm) and following acclimation, activated sludge was added to each microcosm. HBCD was added to replicate test mixtures at a nominal level of approximately 25 µg/kg soil dw and mixed thoroughly into the soil. Biologically inhibited controls were also prepared.

Degradation of HBCD was observed throughout the test period in the viable test vessels (75% decrease over the test period) compared to much lower degradation (3% after 119 d) in the abiotic controls. A half-life of HBCD in the aerobic soil tested was calculated to be 63 d.

While this study does appear to show degradation under the aerobic conditions tested, some issues need consideration. A more thorough analysis is found in Appendix 4. It is not possible to calculate a mass balance. In terms of nominal concentrations, the amount found on day 0 in the viable and abiotic microcosms was 63.6% and 72% respectively. From the data it is unclear whether the low recovery was due to a poor extraction method or otherwise. However, there is a strong linear loss pattern in the viable system compared to the abiotic control, where levels remained consistent throughout the test. This is in contrast to the lower extent of degradation observed in other studies with aerobic sludge, and the lack of degradation observed in the following study using aerobic soil that had not been amended with activated sludge.

The dominant γ-HBCD isomer did not convert to either the α or β isomers in this test system. However, it is unclear whether the test concentration was actually sufficiently high to enable accurate detection of these isomers. No brominated degradation products were detected. The α and β isomers were not detected after day 0 in the viable microcosms. The release of bromine from HBCD could not be quantified (refer to Appendix 4).

The biodegradation of ^{14}C-HBCD was investigated in aerobic soil following OECD TG 307 (Davis et al., 2004) and using a single surface soil. A series of soil microcosms were prepared and dosed with ^{14}C-HBCD at a nominal concentration of 3.04 mg/kg. Controls included a biologically inhibited control (heat sterilised), a benzoate control (mineralisation assay) and a toxicity control (HBCD + benzoate). Degradation was 106.120persistminimal over the 112 d incubation period, and almost all attributed to degradation of the γ-HBCD isomer. In the biologically inhibited control negligible degradation was observed over the study period. Based on this test of one soil type, HBCD is expected to be persistent in aerobic soils.

Results from this study are in contrast to those obtained in the other two studies (Davis, 2003a; 2005). All three studies were performed on soils collected from the same place and the exposure periods were comparable in these studies. The differences were the longer pre-stabilisation period (35 d) used in the Davis et al. (2003a) study as compared to 15 d in the Davis et al. (2004) study, the addition of activated sludge to the microcosms and the use of much higher concentrations in the Davis et al. (2004) to assess the route of breakdown. Higher concentrations appear to result in slower degradation rates and it is also possible that the microorganisms present in the sludge may have affected the degradation rates of HBCD in that study.

Overall, these studies indicate that HBCD is persistent in soil. Conversion of the dominant γ-HBCD to α or β isomers in the soil was not apparent in these experiments, although it is not clear whether the test concentration was sufficiently high to enable accurate detection of these isomers.

Water/sediment systems

Davis et al. (2003b) tested the transformation of HBCD in two aerobic water/sediment microcosms following OECD TG 308 and GLP. Laboratory batch microcosms were prepared with authentic water and sediment collected from two rivers in the eastern United States. Abiotic controls were also prepared. The stock solution was added near the centre of the sediment layer, several millimetres below the surface of the sediment at nominal concentrations of 34 and 60 µg/kg (sediment dry weight) for the Schuylkill River (Valley Forge, Pennsylvania) and Neshaminy Creek systems, respectively. The incubation following addition of the test substance period lasted 119 d.

In the Schuylkill River system, aerobic conditions were maintained in the water throughout the study, while, in the Neshaminy Creek system, conditions were a little more reducing. Little HBCD was found in the water column. In the viable microcosms from both systems, the α and β isomers were not detected after day 0, indicating that the major γ isomer did not convert to the α or β isomers in these systems. Also, no brominated degradation products were detected.

Half-lives for HBCD losses were calculated using the relationship $t_{1/2} = \ln(2)/k$. Loss of HBCD due to biological processes was determined by subtracting the rate of loss measured in abiotic controls from the rate of loss measured in viable reaction mixtures. This resulted in biodegradation half-lives of 11 and 32 d in the Schuylkill River and Neshaminy Creek systems respectively, where the degradation kinetics were performed to day 64 and day 91 respectively. These half-lives compared to abiotic half-lives in the 2 systems of around 187 d in the Schuylkill River system and around 31 d in the Neshaminy Creek system. The results for the viable systems suggest that, in the environment, HBCD will not persist in viable anaerobic sediments.

While not discussed in the test report in any depth, in the background microcosms for the Neshaminy Creek system, an interfering peak was observed in the LC/MS chromatograms, corresponding to γ-HBCD. The concentration of this isomer in the aerobic microcosm was around 19.5 ng/g dw, or over half the nominal concentration added to the test microcosm. This could mean the sediment used for this test system was contaminated. Further, the elevated levels in both systems found at day 119 (compared to the 3 previous sampling times) are of concern, and it may be that the method of extraction was inadequate to properly enable a characterisation of degradation. The results from this study should be treated with some caution, as it is likely the degradation has been overestimated.

The biodegradation of ^{14}C-HBCD was investigated in aerobic and anaerobic freshwater sediments (Davis et al., 2004). The study design was based on OECD TG 308. Laboratory batch microcosms prepared with sediments and associated river water were dosed with ^{14}C-HBCD, with tests performed for both aerobic and anaerobic sediments. The water and sediments were collected from the Schuylkill River. The viable test mixtures contained nominal HBCD at 4.67 mg/kg sediment dw. In addition, biologically inhibited control mixtures (heat sterilised), benzoate control mixtures (to quantify microbial activity) and toxicity control mixtures (benzoate + HBCD to evaluate the impact of HBCD on microbial activity) were maintained. Based on a consideration of measured redox potentials, it appears the study was not performed under fully aerobic conditions and, in fact, conditions were more indicative of anaerobic. In the viable sediments, HBCD decreased from 95% to 53% applied radioactivity (AR) after 112 d. γ-HBCD was the dominant isomer (78%) of the starting material, and was reduced by almost 44% over the course of the study. Three ^{14}C-products with retention times indistinguishable to those observed in the sludge digester studies were detected in the viable sediment reaction mixtures (described further below). Product I (tetrabromocyclododecene, or TBCD) increased to 14% AR after 28 d before decreasing to around 5% by the conclusion of the study. Product II (dibromocyclododecadiene, or DBCD) reached a maximum of 32% after 56 d and remained fairly constant thereafter. Product III (1,5,9-cyclododecatriene, or CDT) was not detected until after 21 d and levels continued to increase through to day 112 (but still <10% AR by this time). $^{14}CO_2$ was <1% AR over the course of the study.

In the biologically inhibited sediment, limited transformation of HBCD occurred (around 15% degradation over 112 d). Over the first 28 d only Product I was detected at around 3% AR, rising to a maximum of 11% AR by day 112. By the end of the study, Product I and II accounted for 100% of the HBCD transformation observed in the biologically inhibited controls.

At the conclusion of the study total recovery of radioactivity was 98% for the viable and 101% for the inhibited sediments. Nearly all radioactivity remained in the sediment layer, with <2% AR typically measured in the aqueous layer. The degradation half-life in aerobic sediment was not determined in this study. However, plotting ln(concentration %AR) against time showed first-order degradation kinetics ($r^2 = 0.78$). A rate constant was determined to be 0.0059 and a half-life (through combined biotic and abiotic routes) determined to be 117 d. This value should be used with some caution as the test was conducted for a period of time less than 1 half-life under these conditions. In addition, redox measurements at the bottom of the water layer during the test do not provide convincing evidence the conditions were aerobic. Thirteen measurements taken over the course of the study were all <63 mV, and most were <50 mV.

Nonetheless, this study is considered to be of good quality. Mass balance results showed good accountability for the test material, all 3 isomers were ably tested due to the higher test concentrations (that were shown to not be inhibitory to soil microorganisms at the test level) and metabolite formation was well described.

ii) Anaerobic

Sludge

As part of a tiered experiment, transformation of ^{14}C-HBCD was determined under anaerobic conditions using activated sludge taken from a municipal wastewater treatment plant (Davis et al., 2004). ^{14}C-HBCD was added to anaerobic reaction mixtures containing digester sludge in a defined mineral medium. Positive, negative and solvent controls maintained. Anaerobic conditions were maintained throughout the test.

The primary degradation for the 3 diastereomers ranged from 67% to 76% after 7 d and 87% to 92% after 60 d. Degradation was also observed in the autoclaved (biologically inhibited) sludge controls, with a decrease in total HBCD levels from 97% at day 0 to 6% AR at the end of the test. This degradation was likely the result of abiotic processes, as methane concentrations in the inhibited control sludge remained <0.5% of total headspace gas over the course of the study. This contrasted with 4% total headspace gas after 34 d being found as methane in the viable microcosm, and 31% after 60 d indicating significant biological activity over the course of the study.

Degradation in both the biotic and abiotic systems was first-order, with faster half-lives found in the abiotic control. Here, a half-life based on the equation ln(2)/k was found to be around 15 d ($k = 0.0459$, $r^2 = 0.97$). This compared to around 23 d in the biotic system ($k = 0.0293$, $r^2 = 0.58$). The implication is that, where conditions are anaerobic, there are strong abiotic factors influencing the degradation of HBCD.

Three ^{14}C-products were detected as the HBCD decreased in concentration. The appearance and concentrations of these differed in the viable reaction mixtures compared to the inhibited controls. In the viable sludge, Products II and III constituted the greater part of the ^{14}C-products, with Product I never achieving levels >3%. Product II reached a maximum concentration of 50% AR after 7 d before decreasing to 24% AR after 60 d. Product III reached a maximum concentration of 13% AR after 28 d and remained relatively steady to the end of the study. In the inhibited controls, during the first week, Product I reached around 30% AR, while the other 2 remained <2%. From then, the level of Product I remained fairly constant, while Products II and III increased to 54% and 12% respectively by 56 d.

Davis et al. (2004) also performed a supplemental anaerobic digester sludge study to facilitate isolation and identification of transformation products. Reaction mixtures were prepared with nominal HBCD concentrations tested of 0, 1, 50, 100 and 500 mg/L. ^{14}C-HBCD in acetone was mixed with HBCD in the bottles and the acetone was allowed to evaporate. Following incubation, the identification of these products was determined through a combination of HPLC/MS and GC/MS analyses. They were identified as tetrabromocyclododecene (Product I), dibromocyclododecadiene (Product II) and cyclododecatriene (Product III).

Gerecke et al. (2006) report the degradation of a technical HBCD mix and three HBCD diastereomers under anaerobic conditions in sewage sludge. Experiments were conducted by adding individual compounds or mixtures to freshly collected digested sewage sludge from a mesophilic digester. In order to study different incubation conditions, nutrients and primers were added to some experimental set-ups. In addition, grab samples from the inlet and outlet of a full-scale anaerobic digester were analysed to verify the results from the laboratory experiments. For all racemic HBCD incubations, no primers were added. Each stereoisomer group ((±)-α-HBCD, (±)-β-HBCD and (±)-γ-HBCD) was added at a concentration of 3.9 nmol (2.5 µg/L). The technical HBCD mixture was added at around 10 nmol (~6 µg/L). For all, nutrients of yeast (50 mg) and starch (20 mg) were added. In addition, the technical mixture had 4-bromobenzoic acid (around 10 nmol) added as a primer.

For all test substances, degradation was fast. The technical HBCD mixture degraded with an apparent pseudo-first-order rate constant of 1.1 ±0.3/d, corresponding to a half-life of 0.66 d. This was not dependent on the presence of additional nutrients. There was a decrease in HBCD concentrations observed in the sterile control and a half-life of at least 35 d was found in this system. The (±)-β-HBCD and (±)-γ-HBCD degraded more rapidly than (±)-α-HBCD by an estimated factor of 1.6 and 1.8 respectively. A 7 h incubation sample from each of the set-ups was subjected to enantio-selective analysis and showed that the enantiomeric fractions for α-, β- and γ-HBCD were essentially 0.5 (i.e. all were racemic mixtures). There was no evidence that the degradation of HBCD was an enantioselective process in the test system used here.

Soils

The transformation of HBCD was determined in anaerobic soils based on OECD TG 307 and GLP (Davis et al., 2003a; 2005). A single surface soil was used. Soil microcosms were prepared and transferred to an anaerobic glove box with an anaerobic atmosphere. After acclimation, activated sludge was added to each microcosm. HBCD was added to replicate test mixtures at a nominal level of approximately 25 µg/kg soil dw and mixed thoroughly into the soil. Biologically inhibited controls were also prepared.

Overall recovery was low in the viable test vessels and this was not addressed by the study authors. Degradation rate constants were determined by subtracting the abiotic rate constant from the viable rate constant. A half-life of 120 d (0–119 d data) was calculated in the abiotic control compared to 6.5 d (0–21 d data) in the viable system.

HBCD degradation products were not detected in extracts of the soils. Unfortunately, no quantitation of bromide released from HBCD could be performed because the levels of bromide present in the background microcosms were 160- to 250-fold higher than the theoretical concentration that could be released in the test vessels from the level of HBCD added.

The results of this test should be treated with caution. There are questions over the actual aerobic/anaerobic nature of the test system. No reason is provided for the low recovery of nominal values. No mass balance is available, making it difficult to "follow" the chemical through the course of the study. Also, the very high levels of bromide present in the background microcosms did not allow quantitation of bromide released from HBCD, further weakening the results.

Water/sediment systems

Davis et al. (2003b; 2005) tested the transformation of HBCD in 2 anaerobic water/sediment microcosms following OECD TG 308 and GLP. Laboratory batch microcosms were prepared with authentic water and sediment collected from 2 rivers in the eastern United States. Anaerobic microcosms were prepared in an anaerobic and pre-incubated at around 23 ^{o}C for 43 to 44 d to allow the microcosms to stabilise. Abiotic controls were also prepared.

The stock solution was added near the centre of the sediment layer, several millimetres below the surface of the sediment at nominal concentrations 63 and 89 µg/kg for Schuylkill River and Neshaminy Creek systems, respectively.

In the viable Schuylkill River system, conditions did not appear truly anaerobic based on redox potential but were still probably reducing and, by day 119 (end of incubation), the system was considered anaerobic. Similarly, in the viable Neshaminy Creek system, conditions were not truly anaerobic from day 7 to day 43. However, by day 119, conditions were truly anaerobic.

Little HBCD was found in the water column. It is noted that the day 0 recoveries were only around 44% nominal in both systems. The reason for these low recovery systems is unclear. The authors suggest this indicates a rapid removal mechanism was operative both biotically and abiotically. However, it could also be an indication of an inadequate method of extraction.

Half-lives for HBCD losses were calculated using the relationship $t_{1/2} = \ln(2)/k$. Loss of HBCD due to biological processes was determined by subtracting the rate of loss measured in abiotic controls from the rate of loss measured in viable reaction mixtures. This resulted in biodegradation half-lives of 1.5 and 1.1 day in the Schuylkill River and Neshaminy Creek systems respectively. These half-lives compared to abiotic half-lives in the 2 systems of around 10.4 d in the Schuylkill River system and around 9.4 d in the Neshaminy Creek system. The results for the viable systems suggest that, in the environment, HBCD will not persist in viable anaerobic sediments.

While not discussed in the test report in any depth, in the background microcosms for the Neshaminy Creek system, an interfering peak was observed in the LC/MS chromatograms, corresponding to γ-HBCD. The concentration of this isomer in the abiotic anaerobic microcosm was around 4.9 ng/g dw, or around 6% of the level added to the test microcosm. This could mean the sediment used for this test system was contaminated.

The biodegradation of ^{14}C-HBCD was investigated in anaerobic freshwater sediments (Davis et al., 2004). The study design was based on OECD TG 308. The water and sediments were collected from the Schuylkill River.

The viable test mixtures contained nominal HBCD at 4.31 mg/kg sediment dw. In addition, biologically inhibited control mixtures (heat sterilised), benzoate control mixtures (to quantify microbial activity) and toxicity control mixtures (benzoate + HBCD to evaluate the impact of HBCD on microbial activity) were maintained.

Based on measurements of redox potential, the system may not have been truly anaerobic, but conditions were still likely to be reducing. In the viable microcosms the total HBCD concentration decreased from 96% to 37% AR after 112 d. Some loss was noted in the abiotic control, with ^{14}C-HBCD decreasing from 112% to 75% AR over the same time period.

Three degradation products were detected and, based on their retention times, were considered the same Products I, II and III found in the sludge degradation studies. In the viable sediments, Product I reached a maximum of 20% after 28 d then decreased to 6% by day 113. Products II and III began to appear after 2 to 3 weeks and steadily increased to 45% and 10% respectively by the conclusion of the study.

In the abiotic control, the appearance of degradation products were noted, with Products I, II and III reaching maximum concentrations of 23%, 10% and 2% AR at the conclusion of the study.

There was no significant production of $^{14}CO_2$ or other volatile products, as the measured concentration of radioactivity in the headspace gas was always <1% AR.

The degradation half-life in anaerobic sediment was not determined in this study. However, plotting ln(concentration %AR) against time showed first-order degradation kinetics ($r^2 = 0.94$). A rate constant was determined to be 0.0092 and a half-life (through combined biotic and abiotic routes) was determined to be 75.3 d.

iii) Summary of biodegradation studies

Biodegradation studies were performed using aerobic and anaerobic soils and water/sediment microcosms. In one experiment, degradation was minimal over the 112 d incubation period in aerobic soil conditions. In anaerobic conditions using activated sludge, the primary degradation of the 3 diastereomers was 87% to 92% after 60 d. Degradation was also observed in abiotic conditions. In the water/sediment systems disappearance half-lives were 11 and 32 d in the aerobic microcosms, 1.1 and 1.5 d in the anaerobic microcosms and 6.9 d for anaerobic soil. No degradation products were detected in the sediment, overlying water or headspace of the microcosms.

One degradation study used ^{14}C-labelled HBCD to identify the potential metabolites. In addition,by using higher concentrations of HBCD, the disappearance of the α- and β-HBCD could also be followed. The test guidelines accept higher concentrations in order to be able to identify metabolites, as long as the substance has no significant influence on the biological activity (OECD TG 307 and TG 308). In this case the concentrations of HBCD used were found not to affect the biodegradability of the reference substance in the samples (sodium benzoate), i.e. there are no indications of an influence of HBCD on the biological activity of the samples.

Degradation rates were slower in the activated sludge samples, and no degradation of HBCD was observed in the aerobic soil microcosms. Tetrabromocyclododecene, dibromocyclododecadiene and 1,5,9-cyclododecatriene were identified as primary biotransformation products, providing evidence that degradation of HBCD in the environment may occur through a process of sequential debromination. Dehalogenation also occurs in abiotic samples, although at a slower rate. In conclusion, data for HBCD suggest that the substance is persistent in sediment. Primary degradation half-lives are relatively long but likely to be less than 365 d. However, ultimate degradation half-lives are likely to be much longer than 365 d based on an extrapolation ratio of 1:4 for a water:sediment biodegradation half-life.

3) Biotransformation

To test if biotransformation by the cytochrome P450 system could explain the observed compositional difference with technical HBCD mixtures (mainly γ-HBCD), a number of in-vitro assays with microsomal preparations of liver were carried out (Zegers et al., 2005). These showed the α isomer was not significantly biotransformed, but the β- and γ-HBCD isomers were significantly metabolised when incubated in the presence of NADPH as an electron donor.

Law et al. (2006a) examined the bioisomerisation potential of HBCD in juvenile rainbow trout (*Oncorhynchus mykiss*). Metabolite formation in the liver and muscle mediated by P450 enzymes was also examined. Fish exposed exclusively to the β isomer showed statistically significant molar amounts of the α isomer ($p < 0.01$) and γ isomer ($p < 0.02$) compared to the controls. This ability to bioisomerise, along with the fact that the β isomer is present in small concentrations in the commercial HBCD mix, helps explain why it is present in relatively smaller concentrations in biota.

Where fish were exposed to the α isomer, no β isomer was detected after the depuration period, while a small amount of γ isomer was found at the end of the depuration period. However, where exposure was to the γ isomer, a linear increase in the α isomer was found over the first 14 d depuration and this isomer was still found after 112 d depuration. No β isomer was found after 112 d depuration and only very small amounts of this isomer were found at other sampling times. This finding indicates that trout have the ability to bioisomerise the β and γ isomers, but the α isomer is more resistant to bioisomerisation in this fish species.

4) Bioaccumulation

HBCD has the potential to be very bioaccumulative based on its properties of low water solubility, molecular weight of 641.7 and LogKow values of 5–6 for the individual isomers. Using the LogKow of 5.62 derived for a commercial HBCD sample, a BCF of 4,240 (logBCF = 3.627) is predicted by EPIWin.

Some test data and monitoring information on levels of HBCD in biota, including magnification through food chains, are available and discussed below. The data support a conclusion that HBCD is bioaccumulative.

(i) Aquatic

Exposure through water

A flow-through bioconcentration test with the rainbow trout (*Oncorhynchus mykiss*) consisting of a 35 d uptake phase followed by a 35 d depuration phase was performed

(Drottar and Krueger, 2000). Exposure was low (0.18 µg/L) or high (1.8 µg/L). The test was performed according to standardised guidelines (US EPA OPPTS 850.1730, ASTM Standard E1022-84, and OECD TG 305) and conducted in compliance with GLP standards.

In the low exposure group, steady state was not reached and, therefore, BCF values for this group may be underestimated. The BCF was therefore calculated on the basis of the day 35 values. In the high exposure group, steady state concentrations appeared to occur around day 21. BCF estimates were therefore calculated to be as follows.

Table 7.8. Estimated BCF values from water concentrations (µg/L), and tissue concentrations (µg/kg)

	Low exposure group			High exposure group		
	Water conc.[1]	**Tissue conc.[2]**	**BCF**	**Water conc.[1]**	**Tissue conc.**	**BCF**
Edible	0.18	1175	6531	1.8	8370	4650
Non-edible	0.18	3731	20 726	1.8	23 158	12 866
Whole	0.18	2355	13 085	1.8	16 154	8794

1) Mean measured concentrations; 2) day 35 mean tissue concentrations.

Kinetic modelling was performed using BIOFAC. The results are presented in Table 7.9. Modelling predicted much higher BCFs for the low exposure group; however, for the high exposure group where steady state did appear to be reached, this model predicted BCF values almost double those determined experimentally.

Table 7.9. Predicted BCF values

	Low exposure group	**High exposure group**
Edible	14 039	9826
Non-edible	30 294	23 303
Whole fish	21 940	16 450

In the low exposure group, levels found after up to 10 d of depuration in fish tissues were approximately the same as found in these tissues at the end of the uptake phase. From there, they slowly dissipated from the fish tissues over the remainder of the study. In the high exposure group, residues in all tissues after 1 d of depuration were around the same as those at the end of the uptake phase. However, residues in the non-edible and whole fish tissues then continued to climb, peaking based on the sampling regime at 10 d into the depuration period. This is despite an apparent steady state being reached during the uptake phase. From there, a slow decline in tissue levels was observed. If the peak depuration day 10 values were used to determine the BCF for the high exposure group, BCF values for non-edible and whole fish would be 18 735 and 11 853 L/kg respectively.

Kinetic modelling using BIOFAC has estimated depuration rate constants and clearance times for the 2 exposure groups as follows.

Table 7.10. Depuration rates for HBCD based on BIOFAC modelling

	Low exposure group			High exposure group		
	Dep. rate constant (/d)	Time to reach 90% steady state (d)	Time to reach 50% clearance (d)	Dep. rate constant (/d)	Time to reach 90% steady state (d)	Time to reach 50% clearance (d)
Edible	0.020	118	35.4	0.037	62.5	18.8
Non-edible	0.023	99.3	29.9	0.035	65.4	19.7
Whole	0.023	101	30.4	0.036	64.1	19.3

In a study on occurrence and bioavailability of HBCD in sediment and fish from the Cinca River in Spain, Eljarrat et al. (2004) considered the bioaccumulation of HBCD by analyzing concentrations in fish correlated to their length. Total levels in fish (23 samples from 4 sites) were analysed. While HBCD was not detected in samples upstream from the heavily industrialised town of Monzon, in sites downstream, HBCD was found at levels in fish up to 1172 ng/g ww. While a correlation between liver levels and length of the fish (age) was not apparent, a positive correlation between muscle levels and length was found ($r^2 = 0.6$). That is, higher concentrations were found in the muscle of older fish and provide further evidence of the bioaccumulation of HBCD.

Exposure through food

Law et al. (2006a) examined the bioaccumulation parameters (depuration rates, half-life and BMF) of individual HBCD isomers in juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were exposed to environmentally relevant doses of the 3 isomers separately through the diet, and bioaccumulation parameters were determined by analysis of muscle tissue concentrations.

None of the diasterioisomers reached steady state during the 56 d uptake phase. Both the β and γ isomers followed first-order depuration kinetics. The depuration of the α isomer showed an initial rapid depuration for the first 14 d, followed by a slower depuration rate of the remainder of the experiment. Both the β and γ isomers were still accumulating after the first 7 d of depuration, after which they slowly began to decline. The reason for this continuing accumulation early in the depuration phase is unclear, but the authors hypothesised that assimilation of these isomers from the gut was slower than that of the α-HBCD isomer.

The following bioaccumulation parameters were found.

Table 7.11. Bioaccumulation parameters of HBCD diastereoisomers

	Depuration rate constant (k_d) (/d)	**Half-life (d)**	**Average assimilation efficiency (%)**	**Bioaccumulation factors (BAF)**
α-HBCD	Not determined	Not determined	31.1	9.2
ß-HBCD	0.0044	157	41.4	4.3
γ-HBCD	0.0048	144	46.3	7.2

Based on the graphical values for depuration of α-HBCD in the report, the depuration half-life over the first 2 weeks of depuration can be estimated to be around 13 d. From then, levels remained relatively constant during the remainder of the depuration phase.

Exposure through sediment

No test data are available.

Food web

Detailed information including sampling regimes and HBCD levels in organisms relating to the following food web studies are presented in Appendix 4. Table 7.12 summarises some of the food web results.

Table 7.12. Summary of total HBCD levels (ng/g lw) in different trophic levels from various food webs, or biomagnification factors

Food web	Detected levels (ng/g lw)			Reference
	Invertebrates	Fish	Aquatic mammals	
North Sea	<100		477–3079	de Boer et al., 2002
Tees	17	291–1036	54–917	de Boer et al., 2002
Western Scheldt	Not detected	230	930	de Boer et al., 2002
Arctic	–	5–25	50–100	Jenssen et al., 2004
Dutch marine	100	375	225[1]	Leonards et al., 2004
	Biomagnification factors			
Lake Ontario		6.3		Tomy et al., 2004
Arctic		10.3		Sørmo et al., 2006
North-East Atlantic		1.2–2.0		Jenssen et al., 2007
Canadian Arctic		2–17		Tomy et al., 2008

1) Tern eggs

As part of their BSEF study, de Boer et al. (2002) considered HBCD behaviour in various food chains, with all demonstrating the biomagnification potential of HBCD.

North Sea food chain: Samples of animals representing different trophic levels (invertebrates, fish and sea mammals) were analysed to determine the environmental occurrence of HBCD. The model of the North Sea food chain comprised benthic invertebrates (sea stars, whelks and hermit crabs), fish (whiting) and sea mammals (harbour porpoises and harbour seals). Mean HBCD concentrations were <100 ng per gram lipid weight (/g lw) at the lower trophic levels compared with 477 ng/g lw in harbour seal and 3079 ng/g lw in harbour porpoise, indicating biomagnification. Analysis of isomer specific concentrations in harbour porpoise showed the α isomer to be completely dominant in the congener profile (56% to 97% of total HBCD). Similarly, for 1 harbour seal sample, the α congener was very dominant (71% total HBCD).

Tees food chain: The HBCD isomer profile was determined in cormorant livers. HBCD isomers were detected, but not all isomers were in all samples. The sum of the 3 isomers varied from 2.2 to 26.4 ng/g ww, with a mean of 16 ng/g ww. The α isomer dominated, ranging from 70% to 100% of total HBCD levels.

HBCD was found in 4 of 5 female porpoise blubber samples, and total HBCD levels (where found) ranged from 54.4 to 917 ng/g ww. In 3 of these samples, the α isomer dominated (~98% to 99% total HBCD levels). However, in one sample, the 3 isomers each accounted for around one-third of total levels.

Three samples of whiting muscle showed various results. HBCD was not detected in one sample and was found at 290.9 and 1036 ng/g ww in the other 2 samples. The α isomer accounted for around half the total levels. The β and γ isomers were each at around 25% total levels in one sample, while, in another, the γ isomer was dominant (~51% total levels), with α and β isomers present at similar levels. Lower levels of HBCD were found in one whole starfish collected in 2001 (16.92 ng/g ww total HBCD), being mainly α-HBCD, while no β-HBCD was found.

Western Scheldt food chain: A sample selection comprising common tern eggs, gudgeon and mysid shrimp (from 2000 and 2001 sampling) were analysed for total HBCD and separate isomers. HBCD was not found in mysid shrimp. The gudgeon had a total HBCD concentration of 49 ng/g ww (230 ng/g lw). The HBCD concentrations in common tern eggs ranged between 35 and 640 ng/g ww (mean 87 ng/g ww), and between 330 and 7200 ng/g lw (mean 930 ng/g lw). The data confirm the relatively strong bioaccumulation potential of HBCD. In the common tern eggs, α-HBCD was strongly dominating. In most samples the ratio α/γ-HBCD was substantially higher than 20, indicating biotransformation of γ- to α-HBCD in biota.

The biomagnification of α-, ß- and γ-HBCD congeners in a pelagic Lake Ontario food web has been studied (Tomy et al., 2004). Samples consisted of lake trout (*Salvelinus namaycush*, a top predator fish) and the following forage fish species: alewife (*Alosa pseudoharengus*), rainbow smelt (*Osmerus mordax*) and slimy sculpin (*Cottus cognatus*). Invertebrate samples included mysids (*Mysis relicta*) and amphipods (*Diporeia hoyi*).

The ß-HBCD isomer was below method detection limits in all samples. Both α- and γ-HBCD were detected in all food web samples (3 trophic levels) and levels were highest in the top predator lake trout samples. There was a clear difference in the relative abundance of the α- and γ-HBCD isomers among the species, with the results indicating an enrichment of α-HBCD at higher trophic levels. There was linear ($r^2 = 0.72$, $p<0.0001$) relationship between the total HBCD concentrations and trophic level, indicating biomagnification of HBCD in the Lake Ontario food web. The trophic magnification factor (TMF) was calculated to be 6.3. Additionally, predator/prey biomagnification factors (BMF) were generally >1 for both the α and γ isomers, ranging from 0.4 to 10.8 for α-HBCD and 0.2 to 9.9 for γ-HBCD.

Jenssen et al. (2004) describe data obtained on bioaccumulation of BFRs in representative species from the Arctic marine food web at Svalbard. HBCD and certain PBDEs were analysed in representative species of different trophic levels: polar cod (*Boreogadus saida*), ringed seals (*Pusa hispida*) and polar bears (*Ursus maritimus*). HBCD concentrations ranged from 5 to 25 ng/g lw in polar cod, 50 to 100 ng/g lw in ringed seal and 15 to 35 ng/g lw in polar bear. The brief report containing these data notes that polar bears appeared capable of metabolising several of the compounds tested for. It appears this was the case for HBCD that accumulated from cod to seal, but it then had lower levels in polar bears.

Leonards et al. (2004) describe data relating to the transfer of HBCD in 2 food chains (common tern and harbour seal) from the Dutch marine environment. HBCD levels increased from invertebrates (mean HBCD 100 ng/g lw) to fish (mean HBCD 375 ng/g lw) but decreased from fish to tern egg (mean HBCD 225 ng/g lw). This is suggested to imply that tern metabolises HBCD. In the tern egg and fish samples, α-HBCD dominated, while the γ-HBCD dominated in the sediment samples.

Sørmo et al. (2006) report work on biomagnification potential in an Arctic marine food chain consisting of 4 invertebrate species: polar cod (*Boreogadus saida*), ringed seals (*Pusa hispida*) and polar bears (*Ursus maritimus*). Based on mean lipid weight findings, HBCD appears to biomagnify from polar cod to ringed seal (BMF = 10.3), with a slight biomagnification from ringed seal to polar bear ((BMF = 1.5).

Law et al. (2006d) examined the extent of contamination and bioaccumulation of BFRs in a Lake Winnipeg food web, including sampling of sediment, zooplankton, mussels and several fish species. BMF values were >1 for several predator/prey pathways for all isomers. In addition, TMFs were calculated to assess the food-web magnification for the entire food web based on the relationship between trophic level (TL) and contaminant concentration. The rank order of TLs was mussel → zooplankton, white fish → goldeye, white sucker → burbot, walleye (top predators). A significant relationship was found for γ-HBCD, suggesting that HBCD is biomagnifying within the food web. The calculated TMF for ∑HBCDs in Lake Winnipeg was 3.1 (2.3, 2.3 and 4.8 for the individual α, β and γ isomers respectively).

Jenssen et al. (2007) undertook a separate study to characterise exposure of HBCD in animals from different trophic levels in North-East Atlantic coastal marine ecosystems. Levels of HBCD generally decreased as a function of increasing latitude, reflecting distance from release sources. HBCD was found in animals from all trophic levels, except for in calanoids at 2 of the 4 locations considered. HBCD was biomagnified from cod to harbour seals at all locations (BMF values of around 1.2 to 2.0 based on mean concentrations).

Tomy et al. (2009) investigated trophodynamics of HBCD in components of a marine food web from the western Canadian Arctic. In terms of mean total HBCD concentrations, biomagnification was observed within the fish food chain, but not higher between fish and ringed seal, or beluga. The most notable difference regarding isomer composition was for beluga, where the α isomer was enriched (accounting for ~90% of the ∑HBCD body burden), relative to its primary prey species, Arctic cod, where the α isomer accounted for only 20% of the ∑HBCD body burden (β: 4% and γ: 78%).

Tomy et al. (2008) assessed potential for trophic transfer in a marine food web from the eastern Canadian arctic. The authors calculate positive biomagnification (BMF >1) for α-HBCD from cod to narwhal (BMF = 4), and cod to beluga (BMF = 2). Positive BMFs for the γ-HBCD isomer are calculated for cod to narwhal (BMF = 17) and cod to beluga (BMF = 7). Regarding trophic magnification, α-HBCD showed an increase in concentration with increasing trophic level, and a TMF of 2.1 was calculated. Conversely, there was trophic dilution of γ-HBCD, with a TMF of 0.5 calculated.

(ii) Terrestrial

As part of an earthworm (*Eisenia fetida*) reproduction study, uptake by worms exposed to treated soil was assessed by measuring tissue concentrations (Aufderheide et al., 2003). After 28 d of exposure, surviving worms were composited by treatment and placed in glass dishes containing wet paper towels to allow the worms to adequately purge their gut contents. Concentrations of HBCD as separate α, β and γ isomers were determined by HPLC/MS. The following soil exposure concentrations (measured, mg/kg dw) and total HBCD concentrations in worm tissues (measured, mg/kg tissue) were found.

Table 7.13. Tissue concentrations and calculated bioaccumulation factors (BAF) in earthworms exposed to HBCD

Soil concentration	**0**	**61.2**	**145**	**244**	**578**	**1,150**	**2,180**	**4,190**
Total HBCD tissue	<0.2	3.40	7.32	16.8	15.3	53.0	71.2	150
α-HBCD %		61.6	62.2	63.8	73.1	54.7	57.7	48.7
β-HBCD %		10.4	10.5	11.4	13.1	11.5	17.0	15.9
γ-HBCD %		28.1	27.3	24.8	13.8	33.8	25.3	35.4
BAF		0.056	0.050	0.069	0.026	0.046	0.033	0.036

These results indicate that HBCD is taken up by worms through the soil. However, the bioaccumulation factors (BAFs) are <1. It is interesting to note the apparent enrichment of the α isomer in the worms, accounting for between 48.7% and 73.1% total HBCD. While the test substance was not characterised for the separate isomer contents, it is expected the γ isomer would dominate (probably around 80% total HBCD) based on other studies. For example, Davis et al. (2004) report a non-labelled HBCD sample from Wildlife International with the same sample reference number as in this test as containing 85.19% γ-HBCD.

Measured levels in biota

Australian monitoring data for measured levels of HBCD in biota are not available. Data presented below are from international sources.

Aquatic

Fish

The following table summarises findings of HBCD in fish samples from various sampling campaigns. Further details on these findings are provided in Appendix 4.

Table 7.14. Summary of total HBCD levels found in fish (ng/g lw)

Location	Fish	Site type	N	Year	Range	Ref
Sweden	Pike	Upstream	3	1995	<100	Sellström et al., 1998
		Outlet	3		<50–<90	
		Downstream	5		4000–8000	
Baltic Sea	Herring	Background	12	1999	4.9–36	Nylund et al., 2001
Sweden	Herring	Coast	6 sites	2002	1.5–31	Asplund et al., 2004
Norway	various	Lake	7–20	1993-2002	90–880	Schlabach et al., 2004
Norway	Cod	River outlet[1]	11	1998	ND-22.7	Bytingsvik et al., 2004
		River outlet[1]	16	2003	ND-56.9	
		Ocean	16	2003	ND-51.2	
		Ocean (pristine)	6	2003	7.67–23.4	
Sweden	Pike/eel	STP outlet			65–1808	Remberger et al., 2004
Switzerland	Whitefish	Lakes	10	2000	25–210	Gerecke et al., 2003
Belgium	Eel	Scheldt Basin	18	2000	up to 33 000	Morris et al., 2004
		Background	3 sites	2000	32–210	
Netherlands	Eel	Rivers	11	1999	12–570	
North Sea	Cod	Ocean			ND-50	
Spain	Fish	River	23		ND-1172[2]	Eljarrat, 2004
Canada	Fish (lake trout)	Lake (Lake Ontario)	4	1979	33	Ismail et al., 2009
			5	1983	28	
			5	1988	18	
			5	1993	32	
			5	1998	25	
			5	2004	16	
USA	Mackerel	Ocean (coastal)	20	2006	23	Shaw et al., 2009
	Alewife		10		7.6	
	Herring		10		14	

1) With input from industrialised areas; 2) wet weight.

In some of these studies, consideration was given to particular HBCD isomers. In Gerecke et al. (2003), isomer distribution was quantified for samples of fish from 4 of the 6 lakes sampled. The α-HBCD isomer dominated and was >85% total HBCD from 3 samples and >58% in the other lake.

In Morris et al. (2004), where HBCD was detected in eel from the Scheldt Basin, α:γ HBCD ratios were around 2.5:3.5, although at one location, a deviating pattern was found, with an α:β:γ ratio 21:21:1. This was the location with the highest total HBCD concentration in eel. At the other location with a relatively high total HBCD, β-HBCD was also found, ratio α:β:γ: 5:1:2. In most other eel samples β-HBCD was not found. In none of the samples was β-HBCD or γ-HBCD higher than α-HBCD.

In the Ismail et al. (2009) study, total levels of HBCD showed a significant, exponential and declining trend during the sampling years. The half-life was in the region of 35 years, although the data were not well correlated. α-HBCD was the dominant isomer. This was also the most recalcitrant in terms of declining levels over the years of sampling, with a half-life in levels from the sampled trout of ~51 years ($r^2 = 0.23$), compared to the low levels of β-HBCD ("half-life" ~13 years, $r^2 = 0.73$) and γ-HBCD ("half-life" ~16 years, $r^2 = 0.58$). The slower rate for α-HBCD may be the result of bioisomerisation of the other 2 isomers, but this is unclear.

Showing the dominance of the α isomer in biota, Janák et al. (2005) report on levels of HBCD diastereoisomers and their enantioner fractions in shrimp and in muscle and liver of various fish species from the Western Scheldt Estuary. Selected sampling sites included 2 locations in the proximity of a HBCD production plant at Terneuzen, the Netherlands (locations 1 and 2, Table 7.14), and 3 locations toward Antwerp, Belgium (locations 3, 4 and 5, Table 7.14). Sampling was originally performed during October and November 2001 to determine PBDE levels. Reversed-phase LC/MS-MS was used for analysis. Muscle and liver of gadoids (whiting and bib) and flatfish (sole and plaice) available from at least 3 locations were pooled according to species and location using 3 to 6 individuals per pool. Muscle of individual eel and pools of shrimps were available from 2 locations. The following results were found.

Table 7.15. Concentrations of alpha- and gamma- diastereoisomers (ng/g lw) in shrimp and fish samples from 5 locations in the Western Scheldt Estuary

			Location				
Species	**Tissue**	**Isomer**	1	2	3	4	5
Shrimp	whole	alpha			28	38	
		gamma			18	<2	
Eel	muscle	alpha			27		7
		gamma			3		2
Sole	muscle	alpha	130	1100	360	110	
		gamma	6	13	17	11	
	liver	alpha	150	680	110	100	
		gamma	<1	<1	8	<1	
Plaice	muscle	alpha		38			
		gamma		<2			
	liver	alpha	23	26		21	
		gamma	4	6		8	
Bib	muscle	alpha	97	53	53		
		gamma	39	43	<3		
	liver	alpha	73	150	100		
		gamma	5	5	10		
Whiting	muscle	alpha		75	45	65	
		gamma		38	<3	51	
	liver	alpha		170	16	240	
		gamma		9	<3	35	

The α-HBCD diastereoisomer was most abundant in all fish samples, with a higher contribution to the total HBCD levels in liver compared to muscle for bib and whiting. The γ-HBCD diastereoisomer accumulated less in liver than in muscle of sole, bib and whiting. A significant enrichment of the (+)α-HBCD enantiomer was found in whiting and bib liver samples.

Marine mammals

The following table summarises some findings of HBCD in marine mammal samples from various sampling campaigns. Further details on these findings are provided in Appendix 4.

Table 7.16. Summary of total HBCD levels found in marine mammals, ng/g lw (bolded values ng/g ww)

Location	Animal	n	Year	Range	Mean	Ref
Baltic Sea	Grey seal	20	1985-2000	16–177	59	Roos et al., 2001
California	Sea lion	1	1993		5.3	Stapleton et al., 2006
		7	1997		3.46	
		8	1997		4.3	
		1	1998		1.1	
		2	1999		4.8	
		4	2000		30.3	
		2	2002		10.8	
		1	2003		17.2	
UK	Harbour porpoise	23	1994–99		113	Law et al., 2006b
		13	2000		148	
		15	2001		1408	
		18	2002		4570	
		16	2003		7422	
UK	Harbour porpoise	16	2003	72–11 500	5450	Law et al., 2008a
		31	2004	19–4150	1360	
		63	2005	<5–8470	1180	
		28	2006	64–6358	817	
Scotland	Harbour porpoise				2900	Zegers et al., 2005
					5100	
Eastern United States	Atlantic white-sided dolphin	57 (blubber)	1993–2004		19–360	Peck et al., 2008
		16 (liver)	1993–2000		3–140	

Analysis on a diastereoisomer basis showed that α-HBCD dominated over the other isomers and was detected in all samples analysed (Law et al., 2006b). Similarly, the isomer composition of HBCD residue in blubbers of a selection of 10 harbour porpoises and 9 common dolphins was shown samples to contain exclusively the α isomer (Zegers et al., 2005).

In the Law et al. (2008a) work, the β and γ isomers were rarely found. A statistically significant decrease in levels was found from 2003–04, compared to the statistically

significant increase from Law et al. (2006b) between 2001 and 2002. This is possibly due to the closure in 2003 of an HBCD manufacturing plant in North-East England which had considerable emissions up to this time.

No increasing or decreasing trends in HBCD concentrations in white-sided dolphin blubber from the eastern USA were observed from 1993 to 2004 (Peck et al., 2008).

Terrestrial

Invertebrates

No data are available.

Birds and mammals

The following table summarises some findings of HBCD in terrestrial birds and mammal samples from various sampling campaigns. Further details on these findings are provided in Appendix 4.

Table 7.17. Summary of total HBCD levels found in terrestrial animals, ng/g lw (ng/g ww in bold)

Location	Animal	N	Year	Range	Mean	Ref
Svalbard, East	Polar bear (fat)	15	1999–2002	18.2–109	44.4	Muir et al., 2006
Greenland		11		32.4–58.6	44.5	
Bering–Chukchi Sea		8 (f)		<0.01–35.1	0.4	
		7 (m)		–	<0.01	
	Birds (eggs)					
North Sweden	Peregrine falcon	17	1991–99	<9–1100	210	Johansson et al., 2009
South Sweden		19	1992–99	<10–2400	270	
U.K.	Peregrine falcon	51	1973–2002	ND–1200		Leslie et al., 2004
Greenland	Peregrine falcon	41	1981–2003	ND–230	28	Sørensen et al., 2004
Sweden	Guillemot	90	1969–79		45–150	Sellström et al., 2003
		100	1980–90		34–130	
		109	1991–2001		81–170	
Arctic (Norway)	Glaucous gulls	31	2006	7.23–63.9	19.8	Verreault et al. 2007
North Norway	Herring gull	10	1983		16	Helgason et al., 2009
		10	1993		31	
		10	2003		108	
	Atlantic puffin	10	1983		12	
		9	1993		32	
		10	2003		58	
	Black-legged kittiwake	10	1983		30	
		10	1993		57	
		10	2003		142	
	Birds (muscle)					
UK	Sparrow hawk	65	1975–2001	ND–19 000		Leslie et al., 2004
Baltic Sea	Guillemot (female)	10	2002		66.7	Lundstedt-Enkel et al., 2005
	Guillemot (male)	10	2002		62.7	
	Birds (yolk sac)					
Norway	European shag	30	2002		417	Murvoll et al., 2006
	Birds (plasma)					
Arctic (Norway)	Glaucous gulls	49	2006	<0.63–6.12	1.73–2.07	Verreault et al., 2007

Leslie et al. (2004) considered concentrations of the 3 diastereomers of HBCD (α, β and γ). All 3 diastereomers were detected in both peregrine falcon and sparrow hawk, although the patterns varied from sample to sample. α-HBCD was found in all but 2 of

the 12 peregrine falcon samples in which HBCD diastereomers were detected. In those 2 samples, β-HBCD was the only diastereomer detected. No α-HBCD was detected in 4 of the 9 sparrow hawk samples. In these 4 samples, either β-HBCD or β- and γ-HBCD was detected.

Of the studies considering the time trend of HBCD in terrestrial biota, those from Leslie et al. (2004) did not suggest a trend of increasing or decreasing residues with sampling year. Data from Sellström et al. (2003) indicated a steady and significant increase in concentrations over time up to the 1992 sampling, but this increase appeared to level out from the mid-1990s. However, Helgason et al. (2009) showed clear increasing trends in the 3 bird species considered from 1983 to 2003. Also in this study, the α-HBCD isomer was present in all samples of eggs, while the β-HBCD and γ-HBCD isomers were not detected.

Conclusion

In summary, a BCF of 18 100 was measured in fathead minnow, exposed to 0.0062 mg/L HBCD for 32 d. Similar BCF values were obtained for rainbow trout. BAFs of 9.2, 4.3 and 7.2 for α-, β- and γ-HBCD, respectively, were calculated by exposing juvenile rainbow trout to the 3 isomers of HBCD concentrations ranging from 12 ng/g to 29 ng/gl weight in the diet. Bioaccumulation of γ-HBCD was linear, while that of α- and β-HBCD increased exponentially, with respective doubling times of 8.2 and 17.1 d. In contrast, BAFs in earthworms (terrestrial BAF), calculated in a reproduction study, were less than one.

Strong positive linear correlation between tissue concentrations of HBCD and trophic level in lakes were reported. Trophic magnifcation factors of 2.3, 2.3 and 4.8 for reported α-, β- and γ-HBCD, respectively, were reported in a Lake Winnipeg food web.

HBCD also undergoes bioisomerisation in organisms, with statistically significant amounts of α-HBCD measured in the muscle tissue of trout exposed exclusively to the γ-isomer. Similarly, both α- and γ-HBCD were present in statistically significant quantities in fish exposed only to β-HBCD. The results suggested that some organisms, such as juvenile rainbow trout, were able to bioisomerize the β and γ isomers of HBCD, with preferential formation of the α isomer. Selective bioisomerisation of HBCD has the potential to contribute appreciably to determining isomer distributions within organisms.

5) Other partitioning behaviour

Likely partitioning behaviour based on physical and chemical properties and the bioaccumulation potential of HBCD have been discussed above. In addition, adsorption/desorption is an important environmental fate partitioning process.

i) Adsorption/desorption

No measured data on adsorption/desorption of HBCD are available. Adsorption to solid surfaces is the main partitioning process that drives distribution in soil, surface waters and sediments. Where K_{oc} is not available, it may be estimated from the K_{ow}. The properties of HBCD indicate that binding to soil will be very strong.

HBCD is a non-ionic substance and the methods for estimating the solid–water partition coefficient is based on standardisation to K_{oc} based on the organic carbon content of different media (soil, sediment or suspended particles). For this assessment, a LogKoc of 5.07 (K_{OC} = 117,600 L/kg, HBCD) has been predicted for soil using the EPISuite of software (EPI, v3.11).

6) Multimedia environmental assessment

Whenever possible, estimation of the overall environmental fate and partitioning of the chemical should be done quantitatively, using appropriate mathematical models (e.g. a generic fugacity model – OECD, 1999). For the purpose of this assessment, the Mackay Level III fugacity model will be used in accordance with OECD guidance (OECD, 2004c). This guidance states that, if possible, this model should be adapted to model the environment of the member country – for example, by using appropriate sizes for the compartments. If this is not possible, the default compartment sizes (EQC standard environment) should be used.

The use of the Mackay Level III model (publicly available from http://www.trentu.ca/cemc/models/models.html along with the Level I and Level II models) is used widely within OECD existing chemicals assessments using the EQC (Equilibrium Criterion) default environment.

i) Transport between environmental compartments

Mackay et al. (1996) outline a 5-stage process for obtaining an understanding of the fate of a substance after discharge to the environment, and for predicting the concentrations to which organisms in various environmental media will be exposed. The 5 stages are shown in Table 7.18.

Table 7.18. Five stages for a multimedia environmental assessment

	Stage	Implementation strategy
1	Chemical classification	From chemical properties, select an appropriate model and identify required data.
2	Acquisition of discharge data	Obtain data on chemical production, use, discharge, and any background concentrations.
3	Evaluative assessment of chemical fate	Deduce the general features of chemical behaviour in a generic environment at 25 ^{o}C.
4	Regional or far-field evaluation	Estimate chemical fate in region of 10^4 to 10^6 km^2 and identify local situations deserving further study.
5	Local or near-field evaluation	Evaluate local behaviour in regions and media of high use and exposure.

Stage 1 – Chemical classification

For running the Level III fugacity model, HBCD has been classified as a type 1 chemical. This chemical type is appropriate for most neutral organics and requires partitioning data for water and fat or lipid solubility, vapour pressure, Henry's Law constant and LogKow. These data are available (or have been calculated) and have been discussed in Section 3.

Stage 2 – Acquisition of discharge data

This information has been described in detail earlier in this Section.

Stage 3 – Evaluative assessment of chemical fate

The default environmental dimensions provided by the EQC model were used and are given in Table 7.19.

Table 7.19. Model environmental dimensions

	Area (m^2)	**Depth** (m)
Volume of air	1E+11	2000
Volume of water	1E+10	20
Volume of soil	Air–Water	0.2
Volume of sediment	= Water	0.05

Multimedia models require half-life data. Half-life data to be used in the modelling have been defined in Section 7.3.3.2. The partitioning results are shown in Table 7.20.

Table 7.20. Level III fugacity model output for HBCD

Compartment of release	**Release**	**Air (%)**	**Water (%)**	**Soil (%)**	**Sediment (%)**
Air	100%	<0.01	<0.01	99.9	0.06
Water	100%	<0.01	0.4	96.0	3.57*
Soil	100%	<0.01	<0.01	99.9	0.05
All equal	33.3% each	<0.01	0.01	99.9	0.13

*The most significant environmental compartment is predicted to be soil, even when release is all to water. However, where release is to water, the sediment compartment will be a more significant sink for HBCD than for other release scenarios. It should be noted that, in the case of total release to water, the model predictions do not seem reasonable, and in reality a much higher amount of HBCD is likely to remain in sediments. However, the fugacity model employed is a box model of an environmental system containing a sediment layer of one cm deep. Therefore, as fresh sediments are layed down, the older sediments are in effect "advected" out of the box, so HBCD associated with them is essentially removed from the system, thereby leading to the model concluding a relatively low overall percentage of HBCD remaining in the sediments.

The implications of this analysis are that, where sewage effluent or biosolids are applied to land, the chemical would associate with the soil, where it would remain tightly bound. Similarly, where disposal of articles containing the chemical occurs to landfill, HBCD would be expected to remain associated with surrounding soil in the event it migrates out of articles, and would remain immobile.

Sensitivity of the model

Changes in model outputs based on differing environmental characteristics were considered. The EQC model as used above assumes water covers 10% of the region's surface. This compares to 3% surface area for water used in the European EUSES model. Reducing the surface area of water from 10% to 3% (keeping all other things equal) did not alter the model outputs significantly indicating the results obtained should be satisfactory for drier Australian environments.

Stage 4 – Regional or far-field evaluation

Stage 4 sees the focus shift from understanding how the properties of the chemical determine its fate to how the characteristics of the specific regional environment(s) of interest will affect fate. Consequently, the regional parameters of the receiving environment should be well characterised to allow modification of the environmental characteristics within the model. Presently, no defined Australian region for use in this model is available, so the evaluative assessment (Stage 3 above) is the only one that can be undertaken at this point using the Level III fugacity model.

Stage 5 – Local or near-field evaluation

The last stage in the multimedia exposure assessment of new substances is conducting a local evaluation, necessary for predicted environmental concentrations (PECs) for media shown to be of concern during the evaluative/regional assessment.

These are calculated below.

7.4 Predicted environmental concentrations

A model for determining local concentrations of chemicals where release is through a sewage treatment plant (STP) is available (DEH, 2003). Local concentrations are determined in receiving waters and soil from application of sewage effluent and sewage sludge. In establishing this model, several Australian specific values were determined through agreement with State and Territory environment agencies.

In addition, the European Union Technical Guidance Document (EC, 2003) provides guidance and formulae (as used in the EUSES model) for determining local concentrations in air, water, sediment, soil and groundwater. These calculations are performed based on simple equilibrium partitioning equations and may provide further useful tools for estimating expected local concentrations.

Before calculating local PECs, a breakdown of emissions is required.

7.4.1 PEC_{air}

HBCD is unlikely to be in the atmosphere in its gaseous phase based on fugacity modelling results and the properties of the chemical. Nonetheless, HBCD has been measured in the atmosphere, most probably bound to aerosol particles. A discussion of measured atmospheric concentrations is found below.

There are no defined scenarios for predicting air concentrations in Australia. Australia's 2 largest cities, Sydney and Melbourne, cover areas of approximately 12 000 km^2 and 7700 km^2 respectively. If the atmospheric height is 2000 m, the atmospheric compartments would be in the order of 2.4×10^{13} m^3 and 1.54×10^{13} m^3 respectively.

Local release estimations indicate that a total of around 300 kg per annum may be released to the atmosphere (see Table 7.6, Section 7.2.4). Assuming processing occurs on 200 d per annum, the daily release is 1.5 kg. Even if all processing occurred within just one of these cities, the daily atmospheric concentration would range from 62.5 to 97 pg/m^3.

While this does not account for accumulation over time, equally, it does not account for atmospheric exchange. In addition, HBCD would mainly (if not totally) be in the atmosphere sorbed to particles, where they would be subject to deposition and therefore removed from the atmospheric column.

Given the majority of HBCD processed in Australia occurred in 1 city (Melbourne), the above assumptions are considered reasonable for this assessment.

7.4.2 PEC_{water}

While the properties of HBCD and modelling outputs suggest low levels of the compound would associate with the aquatic compartment, release to water through STPs from manufacturing operations will occur.

Removal through the STP is predicted based on the SIMPLETREAT model, which predicts removal from water through degradation, volatilisation or sorption to sludge and uses parameters of LogKow (5.62 for the commercial product) and LogH (1.07 $Pa.m^3/mol$). While some degradation may be expected under anaerobic conditions, it will be assumed no degradation will occur during the residence time in the STP. SIMPLETREAT predicts under these inputs that volatilisation to air will be between 1% and 5%, with sorption to sludge of 59% to 85%. Removal in wastewater treatment plants can also be modelled using EPIWIN. Modelling for mixed isomers of HBCD and using measured data for water solubility, vapour pressure and LogKow, this program predicts 0.28% volatilisation to air, 88.65% sorption to sludge, and 0.75% degradation resulting in total removal of 89.68%.

The predicted level of sorption to sludge from EPIWIN corresponds reasonably well with the highest level predicted through SIMPLETREAT, while the level of volatilisation to air predicted through EPIWIN is lower than the lowest level predicted through SIMPLETREAT. For this assessment, the following removal levels through the STP will be used:

Degradation:	0%
Air:	1%
Sludge adsorption:	85%
Total removal:	86%

Table 7.6 (Section 7.2.4) indicates that annual releases to water from all processes is 1150 kg. In addition to the PEC determined for local releases, diffuse releases resulting from service life of HBCD containing products must be considered. Annual release from this life-cycle stage is around 15 kg, resulting in a daily release of around 41 g based on release over 365 d per annum.

When the assessment for HBCD was undertaken, processing of HBCD technical grade granules occurred at one site only in Australia, with ultimate release of water to Melbourne Waters Western Treatment Plant at Werribee STP. Before this, the effluent liquid by-products are collected in a biological treatment plant on-site. The sludge is sent to a composting area to be used as compost later, and the liquid goes to the sewer. Advice received in 2011 is that processing of granules no longer occurs.

Before this, the effluent liquid by-products are collected in a biological treatment plant on-site. The sludge is sent to a composting area to be used as compost later, and the liquid goes to the sewer. Information provided for the assessment indicates that manufacturing of flame-retarded EPS resin occurs over 150 d per annum. With an estimated annual release of 287.5 kg HBCD to water from handling and compounding operations and removal of 86% through sorption to sludge and volatilisation, this equates to a daily release of 270 g to the sewer. The STP plant that receives this release has a discharge of around 485 ML of wastewater per day.[3] It will be assumed no further removal occurs, and the resulting PEC in effluent is 0.56 µg/L, which is below the water solubility of HBCD.

Information provided suggests that the bulk of HBCD as a liquid dispersion used in automotive and technical textiles will be processed at several different sites in Melbourne, where release would occur to Melbourne Waters Western Treatment Plant at Werribee STP. Actual site characteristics for the processing operations are not available, so it will be assumed no on-site wastewater treatment occurs, and all effluent is discharged to the STP. If it is assumed release from such operations occurs over 200 d of the year (average use of 3.0 kg/d) and removal of 86% through sorption to sludge and volatilisation, this equates to a daily release of 420 g to the sewer. Assuming no further removal and the daily discharge of 485 ML from the treatment plant, the resulting PEC in effluent will be 0.87 µg/L.

Where liquid dispersions of HBCD are used in coating polymers for manufacture of sunblinds, processing occurs at one site in Sydney. There are no site characteristics available (such as whether there is on-site wastewater treatment); however, the industry is located in Rydalmere, and it is expected any release to sewer will ultimately be transported to the North Head STP.[4] This plant has a discharge of 336 ML per day. If it is assumed HBCD is used in back-coating operations for 50 d per annum, daily release to the sewer will be 2600 g/d (annual release to water from this operation estimated to be 130 kg). Removal in the STP of 86% will result in a daily concentration in the STP effluent of 1.08 µg/L, which is below the water solubility of HBCD.

For other local operations (conversion of EPS resins containing HBCD or use of HBCD as a liquid dispersion in automotive and industrial textile industries), no actual site data are available. The model outlined in the guidance for estimating predicted environmental concentrations (DEH, 2003), allows release to be distributed over smaller proportions of the population. For the local release estimates, a value of 10% has been chosen – that is, all release will occur to STPs servicing 10% of the population. Annual release to water from these operations is estimated to be 133.5 kg. Assuming such operations occur over 200 d per annum, a daily release of 670 g is estimated. The resulting $PEC_{effluent}$ is estimated to be 0.24 µg/L.

Before finalising expected concentrations in receiving water, a background concentration is derived based on diffuse release estimates. For HBCD, annual release to water has been estimated to be around 15 kg. Assuming that all of this goes through STPs, a background concentration in effluent (based on release over 365 d and 86% removal) is calculated to be 15 ng/L (0.015 µg/L).

[3] http://www.melbournewater.com.au/

[4] (http://www.sydneywater.com.au/OurSystemsAndOperations/WastewaterTreatmentPlants/)

When calculating the PEC of industrial chemicals to surface water, 2 dilution levels are considered. No dilution is assumed for release to inland rivers and a dilution of 10:1 is assumed for initial release into coastal waters. The $PEC_{water\ local}$ values are shown in Table 7.21.

Table 7.21. Summary of PECs in receiving waters

Situation	PEC_{river}	PEC_{ocean}
Plastics industry – processing (1 site only – technical grade granule – Melbourne)	Not applicable	0.06 µg/L
Automotive and technical textiles – processing (several sites, predominantly in Melbourne)	Not applicable	0.09 µg/L
Textile industry – processing, Liquid Dispersion, blinds (1 site only – Sydney)	Not applicable	0.11 µg/L
Other local sources (Plastics industry – EPS resin conversion; Textile industry – automotive and technical textiles)	Not applicable	0.02 µg/L
End use (regional – background)	0.015 µg/L	0.0015 µg/L

7.4.3 $PEC_{sediment}$

The concentration in sediment has been estimated using equilibrium partitioning methodology as described in the EU TGD (EC, 2003) with relevant default values taken from the Level III fugacity model. These default values include a fraction of organic carbon in suspended matter (Foc_{susp}) of 0.2, sediment composition of 20% solids and 80% water, a bulk density of solids of 2400 kg/m^3 and, therefore, a density of sediments of 1280 kg/m^3.

The first step to estimating the $PEC_{sediment}$ is to determine the partition coefficient for solid–water in suspended matter. This is the Foc_{susp} x Koc and results in a Kp_{susp} = 23 520 L/kg. This value represents the concentration of the substance sorbed to solids (mg/kg) divided by the concentration dissolved in pore water (mg/L).

The next step is to convert this value to the whole sediment compartment. In sediment, solids are assumed to account for 20% of the sediment compartment. Therefore, the sediment whole compartment Kp must be converted to account for the make-up of the sediment (80% water, density 1000 kg/m^3; and 20% solids, density 2400 kg/m^3). The whole compartment partition coefficient is the concentration in solids (mg/m^3) divided by the concentration in water (mg/m^3).

Therefore, the $K_{susp\text{-}water}$ is calculated as 0.8 + 0.2 x Kp_{susp}/1000 x 2400 = 11,290 m^3/m^3.

The final step is to convert the PEC_{water} to a $PEC_{sediment}$ based on the $K_{susp\text{-}water}$ partition coefficient, and the density of the sediment compartment (1280 kg/m^3). This is achieved as follows:

$$PEC_{sediment} = K_{susp\text{-}water}/Density_{sed} \times 1000 \times PEC_{water}$$

The results for predicted sediment concentrations are therefore a $PEC_{sediment}$ for local releases summarised in Table 7.22.

Table 7.22. Summary of PECs in sediments

Situation	$PEC_{sediment\text{-}river}$	$PEC_{sediment\text{-}ocean}$
Plastics industry – processing (1 site only – technical grade granule)	Not applicable	0.53 mg/kg
Automotive and technical textiles – processing (several sites, predominantly in Melbourne)	Not applicable	0.79 mg/kg
Textile industry – processing, liquid dispersion, blinds (1 site only)	Not applicable	0.97 mg/kg
Other local sources (plastics industry – ESP resin conversion; textile industry – automotive and technical textiles)	Not applicable	0.18 mg/kg
End use (regional – background)	0.13 mg/kg	0.013 mg/kg

Highly adsorptive substances might not be considered adequately with this approach, as they are often not in equilibrium distribution between water and suspended matter due to their cohesion to the suspended matter. However, they may be desorbed after ingestion by benthic organisms. In the case when release to the surface water predominantly occurs as particles, this calculation may underestimate the sediment concentration (EC, 2003). This will be considered in further evaluation below when comparing the PEC with monitoring data for existing chemicals and, if necessary, in the risk characterisation.

7.4.4 PEC_{soil}

Local concentrations in soil are predicted based on application of sewage effluent or biosolids from STPs to land. The guidance for estimating predicted environmental concentrations (DEH, 2003) provides methodology for calculating these values. Effluent is assumed to be applied at a rate of 10 ML water per hectare per annum and fully mixed within the top 10 cm of the soil. The soil density in the Level III fugacity model will be used for the calculations, namely, 1500 kg/m^3.

The model assumes that the dry weight volume of biosolids production is 100 kg/ML effluent. This is considered a realistic worst case even though actual figures could be much higher depending on the level of treatment an STP is capable of.

To calculate the PEC_{soil} the model assumes 10 tonnes dry weight per hectare per annum is applied to the land. As with water application, it will be assumed that this is fully mixed in the top 10 cm of soil.

Based on local release estimations above to the STP with 85% partitioning to sludge, the lowest concentration in effluent is predicted to be 0.24 µg/L (local operations for conversion of EPS resins in automotive and industrial textile operations, assumes all release occurs to STPs servicing 10% of the population). The corresponding concentration in biosolids is 0.9 mg/kg dw. The maximum concentration in effluent is 1.08 µg/L (use of liquid dispersions in manufacture of sun blinds, release to North Head STP) with a calculated concentration in biosolids of 66 mg/kg dw.

Degradation in the soil is assumed to be negligible. The model predicts that application of effluent and biosolids to land with a mixing depth of 10 cm would result in a one-year concentration of 0.008 to 0.45 mg/kg soil. In cases where agricultural land is irrigated with effluent, 10 years of continual irrigation to the same land area will result in a predicted accumulated soil concentration of 0.016 to 0.072 mg/kg.

7.4.5 Groundwater

The chemical properties of HBCD are such that it would be expected to remain tightly bound to soil and sediment particles and remain immobile. Modelling supports this. As a result, leaching to groundwater is highly unlikely, although there appear to be no measured data available to support this statement.

7.5 Monitoring data

7.5.1 Australian data

No Australian monitoring data for HBCD in air, water or soil are available.

7.5.2 International data

There are some monitoring data available internationally. However, their relevance with respect to local Australian conditions is questionable. Values are reported here for comparison purposes.

1) Air

A detailed description of sampling and levels found is provided in Appendix 4.

i) Outdoor air

Table 7.23. Summary of total HBCD levels found in outdoor air (pg/m^3)*

Location	Site type	Levels (pg/m^3)	Reference
Canadian Arctic	Remote	<1.8	Alaee et al., 2003
Russian Arctic	Remote	<1.8	
Sweden	Point source	13–740	Remberger et al., 2004
	Urban	76–610	
	Factory	1 070 000	
	Remote	<1–280	
East-central USA	Urban	0.9–11	Hoh and Hites, 2005
	Remote	0.6–1.2	
South China	Urban	0.69–3.09	Yu et al., 2008

* Mean values in bolded text.

HBCD was only detected in the particle phase, with 3 of the 7 samples dominated by γ-HBCD, while one had approximately equal amounts of α- and γ-HBCD. Three samples were dominated by α-HBCD. The amount of β-HBCD (6%–17%) was low and not as air variable as the other isomers (Hoh and Hites, 2005).

Data from Yu et al. (2008) indicated that α-HBCD (59%–68%) was the dominant isomer and β-HBCD was a minor isomer in all air samples, which appeared to be different from commercial products. A large but variable percentage of HBCDs (69.1%–97.3%) existed in the particle phase.

ii) Indoor air

Summary of indoor levels of HBCD in homes and offices is provided in Table 5.2. HBCD concentrations ranged from 3 to 6900 μg/kg dust.

2) Water

As part of a wider study, Remberger et al. (2004) sampled leachate from a landfill site for construction and demolition waste, situated around 30 km north of Stockholm, Sweden, and outgoing wastewater from a laundry north of Stockholm. HBCD in the water varied from 3 ng/L at the landfill site to 31 ng/L at the public laundry.

3) Sewage sludgewater

A more detailed description of sampling and levels found is provided in Appendix 4.

Table 7.24. Summary of total HBCD levels found in sludge (ng/g dw)

Location	Sample type	Levels (ng/g dw)	Reference
Netherlands	Influent	ND–570	de Boer et al., 2002
	Sludge	Up to 93	
	Effluent	ND–140	
United Kingdom	Influent	ND–23.6	
	Sludge	531–2683	
	Effluent	ND	
Ireland	Sludge	210–8315	
Sweden	Sludge	3.8–650	Law et al., 2006c

De Boer et al. (2002) provide further details on isomer distribution:

Netherlands: Where influent and effluent levels were measured, α-HBCD accounted for 100% HBCD in the influent of 3 plants and the effluent of one plant. Otherwise, in effluent, γ-HBCD seemed to dominate (up to 100%). In the sludge itself, the γ-HBCD isomer tended to dominate, but, in many cases, significant levels (up to 50%) of α-HBCD were found.

United Kingdom: The highest influent levels found (23.6 μg/kg dw) consisted of around 50% β-HBCD, with around 33.5% and 13.5% α- and γ-HBCD respectively. In sludge, average residues consisted of 17%, 33% and 49.5% α-, β- and γ-HBCD respectively.

Ireland: In Ireland, average sludge residues consisted of around 23%, 30% and 47% α-, β- and γ-HBCD respectively at one site compared with around 31%, 25% and 44% α-, β- and γ-HBCD respectively at a second. However, at the third site, β-HBCD was insignificant compared to around 7% α-HBCD and 93% γ-HBCD.

4) Sediment

A detailed description of sampling and levels found is provided in Appendix 4.

Table 7.25. Summary of total HBCD levels found in sediments (ng/g dw)*

Location (year)	Sample type	Levels (ng/g dw)	Reference
Sweden (1995)	Upstream	ND	Sellström et al., 1998
	Outlet	Up to 11[1]	
	Downstream	230[1]	
	Upstream	2700–7600[1]	
Scheldt Basin (2001)		ND–260	de Boer et al., 2002
Ireland		ND–29.7	
Ireland (2001)	Dublin Bay	ND–10	
United Kingdom	Estuary	ND–511	
(2000–02)	Rivers	6–1678	
Western Scheldt (2000)		ND–128	
Netherlands	Rivers	ND–34	
Sweden	Various	<0.1–25	Remberger et al., 2004
Spain	River	ND–514	Eljarrat et al., 2004
North Sea (2000)	Coastal	ND–6.9	Klamer et al., 2005
USA (Detroit, 2001)	River	ND–3.7	Marvin et al., 2006
Switzerland (2005)	Lake	Up to 54[2]	Bogdal et al., 2008

* Mean values in bolded text.
1) Levels as ng/g IG (ignition loss basis); 2) Levels as ng/g TOC.

Details of these monitoring studies are provided in Appendix 4. Where individual isomers were measured, it was apparent that the γ isomer dominated in sediments, often found at 90% or more of total HBCD. This was not always the case, however, with sampling in the USA, which showed some sediments dominated by the α-HBCD isomer and where seasonal sampling showed significant shifts in the relative ratios of the isomers. The β isomer was consistently detected at substantially lower levels than the other 2.

5) Soil

As part of a wider study on the environmental occurrence of HBCD in Sweden, Remberger et al. (2004) report soil levels taken from different directions at a distance of 300 to 700 m from a plant manufacturing XPS treated with HBCD during a period of 2 weeks a year. Levels found in the soil showed HBCD concentrations from 140 to 1300 μg/kg dw, with concentrations decreasing with increasing distance from the facility.

7.6 Summary of environmental fate

7.6.1 Physical and chemical properties

HBCD is a highly lipophilic molecule (LogKow of 5.62 for the composite product with all isomers having LogKow values >5) with poor water solubility of 3.4 µg/L for the commercial product and individual isomer solubilities of around 49, 15 and 2.1 µg/L for the α-, β- and γ-HBCD isomers respectively. The product has a low vapour pressure (6.27×10^{-5} Pa at 21 °C).

HBCD diastereoisomers interconvert at temperatures >160 °C. Therefore, when analyzing HBCD, the preferred analytical technique is LC/MS/MS as it allows both diastereo- and enantioselective determination of HBCD in environmental samples.

7.6.2 Abiotic degradation

No photolysis data for HBCD are available. The very low water solubility of HBCD makes testing difficult. While there are no hydrolysis data for HBCD, it would not be expected to hydrolyse under environmental conditions. Modelling indicates the compound will persist in the atmosphere if found in the gas phase with a predicted half-life of 51 h.

7.6.3 Biotic degradation

HBCD was shown to not be ready biodegradable in a standard test. While half-lives in environmental media can be estimated based on the results of this study, where reliable measured biodegradation half-lives are available, they should be used instead. Unfortunately, the results from such data for HBCD remain inconclusive. The ready biodegradability result indicates that under aerobic conditions (for example, top sediment layers, soil or aerobic conditions in STPs), HBCD is persistent.

Several studies have been performed considering biodegradation of HBCD under both aerobic and anaerobic conditions in sewage sludge, soil and water/sediment systems. The data show that, particularly under anaerobic conditions, HBCD may degrade. However, there were many uncertainties relating to the interpretation of the results, and any conclusions on the degradation potential of HBCD resulting from these laboratory tests must be treated with great caution.

Several "paired tests" – that is, studies considering both aerobic and anaerobic degradation in test systems such as sludge, soil, or water/sediment systems – were provided. The tests indicated that anaerobic degradation was always faster than aerobic degradation and there appeared to be a level of concentration dependency, with test systems using lower HBCD test concentrations showing faster degradation than those with (much) higher test concentrations.

Degradation in anaerobic sewage sludge and water/sediments followed a sequential debromination via dihaloelimination steps, with a loss at each step of 2 bromines from vicinal carbons with the subsequent formation of a double bond between the adjacent carbon atoms. Following initial degradation of HBCD, tetrabromocyclododecene (TBCD) is formed, followed by dibromocyclododecadiene (DBCD) and then 1,5,9-cyclododecatriene (CDT). Mineralisation was not observed, indicating that the breakdown products themselves are persistent. Such a breakdown pattern was not generally observed under aerobic conditions where HBCD was more persistent in

sewage, soils and water/sediment systems than under similar anaerobic experiments. In one aerobic water/sediment system, this type of breakdown was observed. However, the aerobicity of the test system was doubtful, with redox measurements suggesting conditions may have been reducing.

The half-life in anaerobic sediments was shown to be around 120 d at 20 ^{o}C based on one test (Davis 2004). Where applied to soil amended with activated sludge, HBCD degraded with a half-life of around 62 d. Metabolites and mass balance from this study were not determined, so the results cannot be fully analysed. However, they are curious, as HBCD only degraded slowly in aerobic sludge or in unamended aerobic soil.

Monitoring data for HBCD in environmental compartments provide better evidence of the persistence of the compound in the environment. For example, concentrations measured in sediment cores suggest HBCD is degraded in sediment more slowly than predicted by laboratory studies. The detections in biota and abiotic samples in remote regions further provide strong evidence of the persistent nature of HBCD in the environment.

While the general trend of results showed γ-HBCD dominates in sediments, and α-HBCD dominates in higher trophic level animals, this was not always the case. In some cases, the only isomer found in sediments was β-HBCD, and this isomer was also found to dominate or be present at a significant percentage in some sewage sludge samples. This is puzzling as it is only present in the commercial formulation at low levels (<10%), and has not been identified elsewhere as being formed through rearrangement of other isomers and introduces futher uncertainty relating to the environmental behaviour of HBCD.

Biotransformation of HBCD within organisms is a possible route of degradation, at least for some of the HBCD isomers. For example, trout were shown to have the ability to bioisomerise the β and γ isomers, but the α isomer is more resistant to bioisomerisation in this fish species. Also, biotransformation by the cytochrome P450 system was observed, with active proteins taken from laboratory rats and harbour seals. Assays showed that β- and γ-HBCD isomers were significantly metabolised when incubated in the presence of NADPH as an electron donor, but not the α isomer. Therefore, selective bioisomerisation may play a critical role in the isomer distribution of HBCD in environmental media. Available time trend data are mixed as to whether HBCD levels are rising in wildlife over time.

Despite laboratory data indicating that HBCD has the potential to degrade, monitoring data from sediments and biota provide evidence that HBCD will persist in the environment, including when it enters the food chain (see Section 7.6.4).

7.6.4 Bioaccumulation

HBCD is very bioaccumulative. BCFs in whole fish of 8 800 to 13 000 were determined experimentally in rainbow trout. These BCFs may even be an underestimation, as steady state was not apparent in the low-dose group after 35 d of uptake. Further, steady state did appear to be reached (based on statistical testing of levels) in the high-dose group; however, levels continued accumulating in the test fish well into depuration period, with levels peaking 10 d after exposure ceased. Modelling predictions based on the results of this test suggested higher BCFs of 16 500 to 22 000 for whole fish. In addition, the chemical was shown to persist in the fish with slow depuration half-lives of 19 to 30 d (up to 101 d for the DT90).

This is consistent with the results seen in rainbow trout following dietary exposure to HBCD. Both the β and γ isomers were slowly eliminated from the fish, with depuration half-lives of 157 and 144 d respectively. The α isomer had an exponential uptake curve; it depurated relatively fast for 1 half-life (around 13 d), then the levels remained steady. All isomers accumulated in the fish with biomagnification factors from food to animals of 9.2, 4.3 and 7.2 for the α, β and γ isomers respectively.

In addition, evidence available from measuring HBCD levels in biota supports a conclusion that the substance bioaccumulates and biomagnifies through the food chain. Several field studies considering biomagnification of HBCD through different aquatic food chains show the ability of HBCD to accumulate in predators. Generally in sediments the γ isomer dominates, while in organisms, particularly higher up the food chain, an enrichment of the α isomer occurs with this dominating the isomer profile in many cases.

This is not always the case, and in instances where the isomer distribution was determined, the β isomer was found to be a significant contributor at times.

In terrestrial organisms, higher levels of total HBCD (tens to thousands ng/g lw) have been found in birds of prey and their eggs, and again data indicate an enrichment of the α isomer. Time trend data are mixed as to whether levels in birds are increasing over time.

7.6.5 Potential for long-range transport

Modelling suggests HBCD will have a low potential to reach remote areas through long-range atmospheric transport by comparison with other chemicals known to travel long distances. However, other mechanisms for long-range transport in the environment exist, and the findings of HBCD in air and wildlife in remote areas indicate that such long-range transport cannot be discounted.

7.6.6 Conclusion

Substantial uncertainties surround the fate of HBCD in the environment. While laboratory data support a conclusion that the substance may degrade faster under anaerobic conditions than aerobic, the mechanisms for this are unclear, as these studies often showed very fast abiotic degradation rates under anaerobic conditions with (often) negligible abiotic degradation under aerobic conditions. Further, monitoring data from sediments in the environment (where conditions are most likely to be anaerobic) show a wide range of levels in sediments, although limited time trend data show HBCD levels in sediments that do not support a conclusion for degradation of HBCD under anaerobic conditions.

There are also uncertainties about the actual isomer composition found in the environment. While the γ isomer dominates the commercial formulation, the α isomer tends to dominate the isomer distribution in higher trophic level animals. This is partly explained by bioisomerisation that may result in formation of the α-HBCD from γ-HBCD in certain animals. Alternatively, there may be a preference for uptake of the α isomer. Further, this isomer has been shown to be some 20 times more soluble than the γ isomer, so, where HBCD is available in the water column, it would be expected that α-HBCD dominates.

Of further note is the occasional prevalence of the β isomer. This isomer has been found to dominate or be present in significant (>20%) levels in some sewage, sediment and

biota samples. The reason for this is unclear, as it is not present at these levels in the commercial formulation, and the limited evidence available does not point to γ-HBCD transforming to β-HBCD in animals or by microorganisms.

Clearly the sources and interconversions in the environmental compartments require clarification. There is sufficient evidence, however, to conclude that, under ambient environmental conditions, HBCD can be persistent and is very bioaccumulative.

7.7 Final environmental concentrations for risk characterisation

7.7.1 Air

No Australian monitoring data are available for HBCD in air. In Section 7.4.1, local PEC_{air} values of 62.5-97 pg/m^3 were calculated using worst-case assumptions that all processing occurs in either of Australia's 2 major cities.

International monitoring data are limited. Remote sampling in air over the Canadian and Russian arctic did not detect HBCD (<1.8 pg/m^3). However, high levels were reported in urban air in Sweden (76–610 pg/m^3 in Stockholm) with levels of <1–280 pg/m^3 in more remote Swedish locations. These samples were much higher than levels found in the United States, where sampling in the highly urbanised air of Chicago resulted in a maximum level of 9.6 pg/m^3 (mean 4.5 pg/m^3).

While the calculated levels for Australian air are reasonably conservative, there is limited information for the derivation of a PEC_{air} for the risk assessment. Use rates of HBCD in Australia are well below those of Europe or the United States. However, in an 117.10urban setting, it is difficult to conclude that this could result in substantially lower levels. For the assessment, a PEC_{air} of 97 pg/m^3 will be used.

7.7.2 Water

No Australian monitoring data are available for HBCD in water. Given the properties of the chemical, it is not expected to partition to the water column. Several PEC_{water} values were calculated based on the processing and use pattern of HBCD in Australia and ranged from 0.02 µg/L in coastal receiving waters where HBCD is used as an additive flame retardant in manufacturing EPS resin to 1.08 µg/L in inland receiving waters (see Table 7.20, Section 7.4.2).

These values are all below the water solubility of HBCD, and are therefore considered reasonable. There are no international monitoring data for receiving waters for comparison, and the calculated values will be used in the risk assessment.

7.7.3 Sediment

There are no Australian monitoring data for HBCD in sediments. Several $PEC_{sediment}$ values were calculated in Section 7.4.3 based on predicted water concentrations and using equilibrium partitioning methodology. These values ranged from 0.13 mg/kg in coastal sediments where HBCD is used as an additive flame retardant in manufacturing EPS resin to 9.52 mg/kg in inland sediments (see Table 7.21, Section 7.4.3).

In some European studies, discrepancies between predicted and measured concentrations for several matrices were pretty large. These data suggest that the calculated levels may be significantly overestimated, particularly those for inland sediments where no dilution in receiving waters is assumed. Data from highly

contaminated sites in Europe show dry weight sediment levels from the low µg/kg range to be generally <1 mg/kg dw sediment.

Due to lack of measured Australian data, a conservative approach needs to be adopted for risk assessment. The $PEC_{sediment}$ levels obtained in Section 7.4.3 (Table 7.21), where release to coastal waters was assumed, will be used for all sediment calculations for risk assessment.

7.7.4 Soil

The PEC_{soil} was calculated for the case of agricultural soils amended with sewage sludge. Degradation was assumed to be negligible and a maximum one-year soil concentration of 0.45 mg/kg soil (spreading biosolids plus irrigation) was calculated along with a 10-year accumulation level (as a result of irrigation only) of 0.072 mg/kg soil. In the absence of local or international monitoring data, these levels will be used for the risk assessment.

8. Human health hazard assessment

8.1 Kinetics and metabolism

Limited numbers of animal studies are available on the toxicokinetics of HBCD. Some data are also available on the separate isomers (α, β or γ isomers) of HBCD.

8.1.1 Absorption

Absorption and distribution of HBCD was studied in rats by Yu and Atallah (1980). Eight female and 2 male Sprague-Dawley albino rats weighing 220 to 275 g were administered a single oral dose of $^{14}C\gamma$ HBCD (3.46 mg) diluted 1:10 with 30 mg of technical HBCD. HBCD was first dissolved in 2 mL of acetone, and the resulting solution later mixed with 4.8 mL of olive oil. This ensured that the relatively low dose of HBCD was fully dissolved. The rats were grouped into 5 subgroups, fed *ad libitum* and administered 0.4 mL of the dosing solution (1.93 mg of HBCD) by oral intubation. One female rat was dosed with 0.4 mL of olive oil only, and served as a control. The females were sacrificed 8, 24, 48, and 72 h and the males 48 h after dosing. Urine and faeces were collected daily, blood samples were collected at 0.5, 1, 2, 4, 8 and 24 h after dosing (from Group 4 and 5 animals) and tissue samples (muscle, fat, liver, kidney, heart, spleen, lung, gonads and uterus) were collected at the time of sacrifice. Unchanged HBCD and its metabolites were analysed by liquid scintillation technique and thin-layer chromatography.

Results showed that the absorption from the gastrointestinal tract was rapid with a half-life of 2 h (ka = 0.35 h), while peak radioactivity in blood was reached 4 h after oral dosing. After 8 h, 43% of the total administered dose was recovered in tissues. At 72 h, ≈17% of the given dose was retained in the tissues. As ≈93% of the dose was excreted as "transformed substance: (metabolites or non-identifiable radioactivity), an oral absorption close to 100% was considered (Yu and Atallah 1980).

8.1.2 Distribution

In the above study, 43% of the total administered $^{14}C\gamma$-HBCD was recovered in tissues after 8 h, with the highest activity found in adipose tissue (20%) and muscle (14%) followed by liver (7%), with much lower activities present in the heart, lung, kidney, blood, brain and gonads. At 24 h, 19% of the administered dose was found in adipose tissue, 3% in muscle and 0.8% in the liver. At 48 h, the radioactivity in fat, muscle and liver was 14%, 3% and 0.3% respectively. At 72 h, 14% was still found in fat, but only 2% in muscle and 0.28% in the liver. The volume of distribution was estimated to be 16.8 L/kg, indicating that the absorbed ^{14}C -HBCD was distributed to various tissues, especially adipose tissue (Yu and Atallah 1980).

The concentration of HBCD as individual α, β and γ diastereoisomers in rodent fat tissue was determined in a 90 d oral (gavage) toxicity study (Chengelis 2001). Crl;CD(SD)IGS BR rats were divided into 2 groups with 20 animals per sex, and administered 0 or 1000 mg/kg bw of a suspension of HBCD for 90 d. The dose volume was 5 mL/kg bw/d. Control animals received corn oil only. Two animals per sex per group were killed on days 2, 6, 9, 13, 20, 27, 55, 89, 104 and 118 and blood and mesenteric and/or omental fat were collected and analysed for individual concentrations

of α-, β- and γ-HBCD by liquid chromatography and mass spectrometry. Results indicated that the stereoisomer concentration of the test substance was γ>> β> α, but the concentrations in adipose tissue were, at all times, α>> γ> β. The steady state was reached by study day 55 in males and day 89 in females. However, it was noted that the highest concentration of HBCD was achieved on day 89 in fat tissue in both sexes, with the α concentration being eight to 12 times higher than the γ isomer. The results indicate a 100-fold higher bioaccumulation of α than of the major γ-stereoisomer. On day 89, the concentration of α-HBCD was higher in females than in males, with mean levels of 3101 µg/g fat for males and 4342 µg/g fat for females. The results indicate a half-life of weeks to months for α, β and γ stereoisomers with the longest half-life for the α stereoisomer.

Szabo et al. (2010) studied the absorption, distribution, metabolism and excretion of γ-HBCD in female mice. Mice were given a single dose of HBCD (3, 10, 30, or 100 mg/kg at 10ml/kg) by oral gavage, and urine and faeces were collected daily for 4 d.

For repeated exposure, mice were dosed for 9 d with 3 mg cold γ-HBCD/kg and, on day 10, 3 mg γ-[14C]HBCD/kg was given orally and urine and faeces were collected for 4 d after the last dose (total of 14 d). A single dose (3 mg/kg) was also administered intravenously via the tail vein at a dosing volume of 2 ml/kg.

Animals were euthanised by CO2 asphyxiation followed by exsanguination via cardiac puncture. Tissues were collected and weighed: blood, bile, liver, lung, kidneys, skin, adrenal glands, urinary bladder, spleen, thymus, adipose (abdominal), muscle (abdominal) and brain. Bile was removed directly from the gallbladder using the BDUltra-fine Insulin syringe. Tissue distribution of γ-HBCD was analysed 4 d after the administration.

All tissues examined had low but measurable levels 4 d after dosing. Tissue disposition was independent of dose for the 3, 10, 30 and 100 mg/kg doses. The largest percentage of the dose was localised in the liver and ranged between 0.21% and 0.29%. This was followed by skin (0.17%) and muscle (0.10%). Low levels were detected in the blood (0.09%), brain (0.01%) and fat (0.005%). These results also demonstrate the absence of tissue-specific sequestration.

Tissue disposition was not altered after a 10 d repeated exposure in all tissues except for adipose and blood. Disposition was significantly increased between a single and repeated oral exposure of 3 mg/kg γ-[^{14}C]HBCD in the adipose tissue (0.005% versus 0.010%, respectively) and in the blood (0.081% vs 0.134%).

The 14 d time-course observation indicated a biphasic profile with an initial steep decline from 1 h to 2 d and a less steep decline between 2 and 14 d.

After oral exposure to γ-[^{14}C]HBCD, the radioactivity was rapidly cleared from the tissues (liver, blood, fat, skin and muscle), so that, by 1 h after oral administration, less than 1% of the dose remained in the tissues. From this point on, a biphasic decay was observed.

8.1.3 Metabolism

Information on HBCD metabolism is extremely limited. In the rat study by Yu and Atallah (1980), elimination of HBCD and its metabolites occurred mainly in faeces (77%) for the initial period of 3 d. About 28% of the excreted radioactivity consisted of HBCD metabolites, while the remaining 72% was not extractable by organic solvents

and was considered to consist of covalently-bound metabolites. Three metabolites were resolved by thin-layer chromatography (TLC) but were not identified.

In this study, the fate of ^{14}C-HBCD administered in rats could be represented by a two-compartment open-model system, the central compartment which consisted of blood, muscle, liver, kidney and other non-fatty tissues, and the peripheral compartment which consisted of fatty tissues. HBCD was rapidly metabolised in the central compartment. The model was useful in predicting the residue level in blood and fat tissue (Yu and Atallah 1980).

8.1.4 Elimination and excretion

Several studies estimating the amount of HBCD excreted in urine and faeces have been published. Although all the studies are not available, references and minimal data from these studies presented in a publication by Hakk and Letcher (2003) are included in this section.

In a study conducted by Dean and Leong (1977)*, in rats administered 7 to 9 mg/kg HBCD as a single oral dose, 70% was excreted in the faeces, and 16% in the urine. However, in an unpublished study by Ryuich et al. (1983)*, where ^{14}C-HBCD was administered daily for 5 d (animal not known) at 500 mg/kg, there was no detectable urinary excretion over 96 h, with the average daily faecal excretion ranging from 29% to 37%. In another unpublished study (Commission on Life Sciences 2000)*, when HBCD was administered to male Wistar rat at 500 mg/kg/d for 5 d, 32% to 35% was excreted in the faeces, with no HBCD detected in the urine.

Following the administration of 7 to 9 mg/kg of ^{14}C-γ-HBCD to 8 female and 2 male Sprague-Dawley rats, it was noted that, based on a two-compartment open-model system (central and peripheral), elimination of HBCD in the faeces after 48 h was faster in males (94%) than females (54%). Disappearance of radioactivity was slower from the peripheral compartment (body fat, k= 0.03 h^{-1}) than from the central compartment (blood, muscle, liver or kidney k=0.17 h^{-1}). At 72 h, 77% of HBCD and its metabolites were excreted in the faeces and 16% in urine, with excretion faster in males than in females. In the faeces about 28% of the radioactivity was in the form of extractable metabolites (4% free metabolites, 7% acid-released, 14% base-released and 3% water soluble) while the remaining 72% was not extractable by organic solvents (ethyl acetate). In urine, 64% of the radioactivity was metabolites while the remaining 36% of urinary ^{14}C was not identified, as it was not extractable from urine. The 3 metabolites resolved by thin-layer chromatography were not identified (Yu & Atallah 1980).

A fine suspension of 500 mg/kg HBCD (Pyroguard SR-103; Daiichi Kogyo Seiyaku KK) was administered by oral intubation to 4 male Wistar rats, each weighing 260 to 300 g for 5 consecutive days. The total dose volume administered was 100 mg/mL. The animals were kept separated in glass cages and urine and faeces were collected daily. Twenty-four hours after the last administration, the rats were sacrificed and the spleen, liver, pancreas, kidneys, heart and fatty tissue collected. The limit of quantification was 5 µg/mL in urine and about 20 µg/mL in homogenates of the biological samples (faeces and various organs with added water 4 times in volume). The average daily rate of excretion in the faeces was 29% to 37% of the daily administered amount in the 4 rats. The cumulative faecal excretion was about 32% to 35% of the total administered dose. Urinary excretion of unchanged HBCD was not observed. No metabolites were found in the urine or faeces. A study of an isolated loop of the upper jejunum indicated that about 12% of the dose was detectable in the intestinal tissue, and the amount remaining in the

lumen of the loop negligible. The test substance was detected in adipose tissue at 0.3 to 0.7 mg/g fat only after dosing for 5 d (*Arita et al. 1983).

Geyer et al. (2004) estimated a HBCD terminal elimination half-life of 64 d for humans by 2 different methods. One method involved using a linear one-compartment open pharmacokinetic model based on body burden and daily intake in humans, while the other estimation method was from the terminal elimination half-lives in rats. In the pharmacokinetic model estimation, the bioavailability is 100%, and the lipid mass is 13.5 kg for males and 18.7 kg for females.

In the study by Szabo et al. (2010) the major route of elimination of HBCD was via the faeces, as evident from the analyses of faeces and urine 14 d after the administration of ^{14}C - γ-HBCD in mice (nearly 55% in faeces and 30% in urine). Approximately 80% of the administered dose had been eliminated in the urine and faeces, collectively, by the fourth day. Expressed as per cent dose, the data demonstrated a lack of dose dependency in excretion. Essentially, no radioactivity was detected in the urine or faeces past day 5.

The amount of ^{14}C derived radioactivity in the urine eliminated on the first day was relatively constant between the intravenous and oral routes of administrations, implying that γ-[14C]HBCD derived radioactivity was absorbed and eliminated similarly after exposure by these 2 routes.

Of the γ-HBCD derived radioactivity eliminated in the urine, 100% was in the form of metabolites. The same was true for the radioactivity detected in the bile and blood. Faeces and liver contained both parent γ-HBCD and metabolites (94% to 96% metabolites and only 4% to 6% parent γ-HBCD). Of the metabolites present, 11% to 15% constituted stereoisomerization products. β-HBCD and parent γ-HBCD were detected in the liver, while the 3 major diastereoisomers – α-, β-, and γ-HBCD – were present in the fat and faeces. These results suggest that intestinal flora may alter the biliary metabolites. The excreted metabolites were not identified in this study.

A distinct observation was the shift from the predominance of γ-HBCD in the commercial mixture and environment relative (>70% γ-HBCD diastereoisomer) to α-HBCD in biota (Law et al. 2008b). This phenomenon can be explained by either a difference in pharmacokinetic rates, where the γ-diastereoisomer is metabolised and eliminated at a more rapid rate than the α-diastereoisomer, and/or stereoisomerisation of the γ-diastereoisomer to α. Selective metabolism is supported by in-vitro assays with phenobarbital-induced hepatic rat and non-phenobarbital-induced seal microsomes, where the γ and β diastereoisomers were significantly metabolised, while the α diastereoisomer was not (Zegers et al. 2005; Covaci et al. 2006). Results from this study further suggest the occurrence of cytochrome P450 (CYP) mediated metabolism.

8.1.5 Dermal absorption

The EU RAR (2008) reported an in-vitro HBCD dermal absorption study using human skin (Roper 2005).* The study was performed according to OECD TG 428. A 30 µl aliquot of ^{14}C-HBCD (corresponding to 640 µg HBCD) was applied to the human breast skin placed in a flow-through diffusion cell apparatus. Receptor fluid was collected hourly and mixed with scintillation fluid for analysis. At 24 h post-dose, the stratum corneum was removed with 20 successive tape strips and analysed by combustion/liquid scintillation counting. The first 5 tapes were pooled and analysed together. This was repeated for tapes 6 to 10, 11 to 15 and 16 to 20.

The results were based on 9 samples of skin obtained from 6 different donors. The cumulative absorption data indicated that the substance had a lag-time of 4 h, implying that absorption could continue for a long time. However, due to limited "lifetime" of the skin in the in-vitro assay, it was not possible to measure for longer than 24 h.

The stratum corneum contained 31.5% of the applied dose. Most of this (25.7%) was recovered in the first 5 tape strips, indicating that the 14C-HBCD was on the surface of the skin and that there was a slow transport over the stratum corneum. Given the low solubility of the substance in the aqueous receptor fluid, only 1 dose level studied, and indications from the cumulative absorption that the substance had a considerable lag-time, the authors considered it appropriate to include strips 11 to 20 in the absorbed dose. The total dermal absorption was thus estimated to be 4% (representing 1.5+1.1+1.3+0.01% from strips 12 to 20 + exposed skin + receptor fluid).

Table 8.1. Distribution of radioactivity (% applied dose) at 24 h post-dose following topical application of HBCD to human split-thickness skin

	Mean	**SD**
Dislodgeable dose	63.4	12.1
SC 1–5	25.7	9.31
SC 6–11	3.12	1.54
SC 12–15	1.54	1.05
SC 16–20	1.13	0.49
Unexposed skin	0.45	0.23
Exposed skin	1.34	0.49
Receptor fluid	0.01	0.00
Receptor rinse	0.00	0.00
Mass balance	96.7	4.25

SC = stratum corneum.

Based on this study, the dermal uptake was assumed to be 4%. The EU assessment has considered a 2% dermal absorption for granules with a mean particle size of >100 µm (EU RAR 2008).

8.2 Effects on laboratory mammals and other test systems

8.2.1 Acute toxicity

Oral

In a limit test conducted on Charles River CD strain of rats (5/sex/group) (Wilson & Leong 1977), HBCD suspended in corn oil was administered orally by gavage at 10 000 mg/kg bw. The rats were observed during the first 4 h after dosing, at 24 h and then daily for 14 d for toxic signs and mortality. Non-lethal signs such as diarrhoea and hypoactivity were noted in 1 out of 5 females, and hypoactivity, corneal opacity and ptosis in 3 out of 5 males. No mortality was reported at this dosage. The LD_{50} was determined to be >10 000 mg/kg bw.

In another limit test (Lewis & Palanker 1978b), 10 g/kg technical HBCD ("GLS-S6-41A") in corn oil was administered to Wistar rats (5/sex/group) by gavage. The observation period was 14 d, during which no toxic or gross pathological changes were observed. One male died on day 6; however, the cause of death could not be ascertained. The oral LD_{50} was determined to be >10000 mg/kg bw.

In an acute toxicity test (Ogaswara et al. 1983), HBCD ("Pyroguard SR-103") suspended in a 1% carboymethyl cellulose (CMC) aqueous solution was administered on days 0, 2, 4 and 6 to 2 groups of CRJ:SD-male SPF 5-week old rats in 2 dosages – 10 000 and 20 000 mg/kg bw – with 10 animals/dose, and one control group. The general condition and mortalities were noted 1, 3, 6, 10 and 16 h after administration and then for 14 d. The bodyweight was measured every day up to the day 10 and day 14, following which all animals were sacrificed. Yellow excreta was observed in both groups on the day after administering the dose but disappeared on the 8th day, and the animals recovered. The yellow excreta was considered to be due to the administration of a large dose of the test compound. A slight, statistically-significant inhibition of bodyweight gain was observed in both groups, and the control group up to the 8th day in several cases; however, the mean bodyweight showed normal steady increases during the study term. None of the animals died and no abnormalities were noted at autopsy in both groups. The LD_{50} was determined to be >20 000 mg/kg.

An acute toxicity test with HBCD was conducted on groups of 10 B6C3F1/S1c male and female mice, administered HBCD in a solvent (*Tobe, 1984). The test substance was suspended in 5% CMC or olive oil to obtain 30% suspensions. The doses were 10 000, 15 000 and 20 000 mg/kg bw/d HBCD in the CMC suspension and 20 g/kg HBCD in olive oil, administered once by gavage. Control groups received 5% CMC or olive oil in the same volumes as those used for the 20 000 mg/kg bw/d HBCD groups. Both males and females in each group exhibited depressed activity (spontaneous motion inhibition) and closed eyes immediately after administration, but recovered 30 minutes later. The test substance was found to be excreted from the rectum 1 h after administration. There was no effect on bodyweight during the 14 d observation period for both males and females, no mortalities and no significant autopsy findings at the highest dose of 20 000 mg/kg bw. The LD_{50} was determined to be >20 000 mg/kg bw.

Dermal

An acute dermal toxicity study was conducted by Wilson & Leong (1977) on New Zealand white rabbits (2/sex/group). HBCD ("Firemaster 100") was applied once at a dosage level of 20 000 mg/kg bw/d to the shaved back under an occlusive dressing. Twenty-four hours later, the bandages were removed, and the backs were washed. No toxic effects were noted, and none of the animals died. The dermal LD_{50} was determined to be >20 000 mg/kg bw.

Following a 14 d range finding study, New Zealand albino rabbits (3/sex/group) were administered 8000 mg/kg bw HBCD ("GLS-S6-41A") as a single dermal application under an occluded patch. The animals were observed for signs of toxicity at 1, 3, 6 and 24 h post-administration. Observations were made daily thereafter for a total of 14 d. No toxic effects were reported, and none of the animals died. The dermal LD_{50} was determined to be >8000 mg/kg bw (Lewis & Palanker 1978a).

Inhalation

In an acute inhalation study (Wilson & Leong 1977), Charles River CD rats (5/sex/group) were exposed whole body to HBCD dust identified as "Firemaster 100" at a concentration of 202 mg/L for 4 h. In the first 10 minutes of exposure an increase of preening activity was noted. Ninety minutes after the start of exposure to 4 h later, slight dyspnoea was noted in the rats. No toxic effects were noted in the following 14 d, and no mortality was reported. The particle size was not reported.

In a second study (Lewis and Palanker 1978a), Wistar rats (5/sex/group) were exposed whole body to HBCD dust identified as "GLS-S6-41A" at a concentration of 200 mg/L for 1 h. At the end of the 1 h period, the animals were returned to their individual quarters and observed daily for 2 weeks. No toxic effects were noted in any of the animals at the end of the observation period. No mortality was recorded. No changes were noted at autopsy. The particle size was not reported. The results of the acute toxicity studies by the various routes are summarised in Table 8.2.

Table 8.2. Summary of the acute toxic effects of HBCD

Animals	Route	Dose	LD50/LC50	Reference
Charles River CD rats	Oral (gavage)	10 000 mg/kg bw	>10 000 mg/kg bw	Wilson & Leong 1977
Wistar rats	Oral (gavage)	10 000 mg/kg bw	>10 000 mg/kg bw	Lewis & Palanker 1978b
CRJ:SD–male SPF rats	Oral	10 000 and 20 000 mg/kg bw	>20 000 mg/kg bw	Ogaswara et al. 1983
B6C3F1/S1c mice	Oral (gavage)	10 000, 15 000 and 20 000 mg/kg bw/d	>20 000 mg/kg bw	Tobe et al. 1984.
New Zealand albino rabbits	Dermal	20 000 mg/kg bw/d	>20 000 mg/kg bw	Wilson & Leong 1977.
New Zealand albino rabbits	Dermal	8000 mg/kg	>8000 mg/kg bw	Lewis and Palanker 1978a.
Charles River CD rats	Inhalation	202 mg/L for 4 h	>202 mg/L	Wilson & Leong 1977
Wistar rats	Inhalation	200 mg/L for 1 h	>200 mg/L	Lewis and Palanker 1978a.

It was concluded that the substance had a very low acute toxicity by the oral, dermal and inhalation routes of administration. LD_{50} is higher than 20 g/kg bw by both oral (rats) and dermal (rabbits) routes. The acute inhalation toxicity is also low, with LC50 greater than 200 mg/mL in rats.

8.2.2 Skin and eye irritation

Skin irritation

A study to determine the skin irritant effects of HBCD was conducted on New Zealand white rabbits (3/sex/group) (Wilson & Leong 1977). The hair was removed from the back of each rabbit, and 0.5 g HBCD identified as "Firemaster 100" was applied to the abraded skin of 3 rabbits and the intact skin of the other 3 rabbits, under occlusion for 4 h. The skin was examined for signs of irritation at 4, 24, 48 and 72 h according to OECD guidelines. Barely perceptible erythema was noted in the intact skin at the end of 24 h in 2 females only, and at the end of 48 h in one female only. No effects were noted in the abraded skin in any of the animals at any period.

In a dermal irritation study 6 New Zealand rabbits were administered 0.5 mL (0.5 g) of technical HBCD (GLS-6-41A) to shaved intact and abraded skin under occlusion for 4 h. The occlusive wrapping was removed 24 h following application and the sites examined and scored separately for erythema and oedema at 24 and 72 h. No irritant effects (erythema or oedema) were observed in any of the animals at the end of the observation period. According to the study report, the test method was considered to be that of Draize et al., however no observations were made beyond 72 h. A dermal corrosion study (Lewis & Palanker 1978b) was conducted using the same doses and similar methodology as the above study except for the observation period (4 and 48 h post administration). No irritant effects (erythema or oedema) were observed in any of the animals at the end of the observation period.

Eye irritation

An eye irritation study was conducted on New Zealand white rabbits (3/sex/group) (Wilson & Leong 1977). HBCD ("Firemaster 100") at a dosage of 100 mg was placed into the conjunctival sac of the right eye. The left eye of each rabbit was used as a control. Observations were conducted at 24, 48, 72 h and 7 d following instillation. Fluorescein application was used to assess corneal effects. No effects on the iris or cornea were seen. The group average scores based on the effects on the cornea, iris and the conjunctiva combined were 0.6, 1.0, 1.0 and 0 respectively at the different time points. The effects noted were mainly conjunctival effects. Based on the scores obtained, HBCD was not considered to be a primary eye irritant.

A test for acute eye irritation or corrosion was conducted with HBCD (FR-1206) on a group of 6 rabbits according to OECD TG 405 for Eye Irritation/Corrosion. One hour after instillation of the test material, slight conjunctival redness (Grade 1) was observed in 5 rabbits and mild conjunctival redness (Grade 2) observed in one rabbit. Slight chemosis (Grade 1) was observed in 4 rabbits, and 2 rabbits showed moderate chemosis (Grade 2). Twenty-four hours after instillation, 2 rabbits showed slight redness. Chemosis was noted in one rabbit which persisted for 48 h. Corneal opacity covering up to a quarter of the cornea (Grade 1) was observed in one rabbit 24 and 48 h after instillation of the test material. Based on these observations, HBCD was not determined to be an eye irritant (Crown et al. 1984 in the LSRI Report No. DSB/060/FR, 1984 (microfiche printout)).

In a study reported by Lewis and Palanker (1978b) 6 New Zealand rabbits (3/sex/group) were administered 0.1 mL of HBCD (GLS-S6-41A) as a single dose to one eye, while the other eye was used as a control. Their eyes were not washed for 24 h. Following application of the test substance, the test and control eyes were examined for signs of irritation at 24, 48 and 72 h and on days 4 to 7, and the effects on the cornea, iris and conjunctiva noted. The results were graded according to the OECD Ocular Grading System (TG 405).

Slight corneal opacity lasting up to 48 h from time of instillation was seen in one animal. Two animals showed slight iris lesions at 24 h, and a separate animal showed slight iris lesions at 72 h. Conjunctival effects at Grade 1 to 2 were seen in 4 out of 6 animals and in one animal Grade 1 chemosis persisted to 72 h.

Based on the results of the Ocular Grading System, the mean eye irritation score at the end of 72 h for all animals was as follows:

Corneal opacity	0.1
Iris lesion	0.1
Conjunctival redness	0.4
Chemosis/conjunctival oedema	0.4
Conjunctival discharge	0.15

HBCD was not considered to be an eye irritant.

8.2.3 Sensitisation

Skin

In a modified guinea pig maximisation test (Nakamura et al. 1994), groups of 10 female Hartley albino guinea pigs were administered 0, 5000 or 50 000 ppm (0%, 0.5% or 5% w/v) HBCD, in olive oil, intra-dermally and 250 000 ppm (25% w/v) in petrolatum as topical application on shaved skin, for the induction phase. Three weeks after the intra-dermal injection, 0, 500, 5000 or 50 000 ppm HBCD in acetone was used in an open patch test on shaved skin as a challenge. At the highest concentration of induction and challenge, 9 out of 10 animals were sensitised, demonstrating a clear dose-effect relationship.

In a study translated from a Japanese journal, Momma et al. (1993) determined the primary irritation, sensitisation, phototoxicity and photosensitisation effects of HBCD on guinea pigs. In the following discussion, only the sensitisation potential of HBCD is addressed. The test was carried out according to the guinea pig maximisation test of Magnusson and Kligman (1969). In the study, groups of 10 female Hartley albino guinea pigs were administered 0.05 mL of 0%, 0.05%, 0.5% and 5% HBCD in olive oil, intradermally, as an induction dose. Seven days later, filter paper coated with 25% HBCD in vaseline (0.2g) was applied as a topical application for 48 h over the area where the injections were given. Thirteen days after the topical induction dose, 0.02 mL of 0.005%, 0.05%, 0.5% and 5% HBCD in acetone was used as a challenge dose applied in an open patch dose on shaved skin and retained for 24 h. In guinea pigs induced with HBCD solution, positive reaction was observed 48 h later with an induction dose of 0.5% or more and a challenge dose of 0.05%. The rate of positive response was correlated with the sensitisation concentration. The degree of skin reaction was slight erythema. At the highest concentration of induction and challenge 9 out of 10 animals were sensitised with HBCD (EU RAR 2008; Momma et al. 1993).

A study (Wenk 1996) was conducted to assess whether a batch of HBCD marketed in the EU caused delayed contact hypersensitivity in guinea pigs as measured by the Magnusson and Kligman Maximisation method. The study was conducted in compliance with the GLP standards of OECD and the US EPA. Although not stated by the authors, the study also conformed to OECD TG 406. Phase 1 of the induction phase

consisted of the administration of 3 pairs of intradermal injections to control (10 animals) and test animals (20 animals). The first pair of injections consisted of 50:50 solutions of corn oil and Freund's Complete Adjuvant (FCA), the second pair consisted of HBCD at a concentration of 5% in corn oil and the third pair of injections consisted of HBCD at a concentration of 5% in the 50:50 corn oil/FCA. In phase 2, after 7 d, the test animals received topical applications of 0.5 g of the neat test material, moistened with corn oil, in an occlusive chamber, at the intradermal induction site. Following a 2-week rest period, the challenge dose (0.5 g HBCD moistened with corn oil) was administered at a separate site to both groups (24 h exposure) using the same procedure as the topical induction. Approximately 21 h after the exposure period, the challenge area was examined and the animals scored according to Draize at 24, 48, 72, 96 and 120 h. Examination of the sites indicated that HBCD produced no erythema or oedema in any animal at 24, 48, 72, 96 or 120 h. Based on the above results, HBCD was considered to be a non-sensitiser.

Wolhiser and Anderson (2003) investigated the contact sensitisation potential of HBCD using the Local Lymph Node Assay (LLNA). The study was conducted in 2 parts: the first part was a primary irritancy study (ear swelling response) which was conducted to determine the HBCD concentrations to be used in the LLNA study. In the primary irritancy study, eight-week-old CBA/J female mice, (2 animals per group) were administered 25 µL/ear of HBCD in N, N-dimethylformamide (DMF), or DMF alone, on the dorsal surface of each ear for 2 consecutive days. HBCD concentrations in DMF were 1%, 5%, 10%, 25% and 50%. Prior to the administration, and on day 3, i.e. 24 h after the final exposure, the thickness of each ear was measured with a digital micrometer. Erythema evaluations of the ear surfaces were not assessed, because the ears of the CBA/J mice are brown. The ear swelling response for each ear was calculated using the following equation:

Per cent ear swelling = B-A x 100/A where A= mean of pre-treatment measurement (mm x 10^{-2}), and B= mean of post-treatment measurement (mm x 10^{-2})

Mean (2 ears) per cent ear swelling was 1.0, 2.0, 6.2, 19.8, 26.5, 19.8 with the vehicle and 1%, 5%, 10%, 25%, and 50% HBCD groups, respectively.

For the LLNA study, 30 mice, 6 in each group, received HBCD at either 2%, 20% or 50% concentrations, or DMF, on days 1 to 3. As a positive dermal sensitisation control, α-hexyl cinnamalaldehyde (HCA) at 30% v/v was used. On day 6, all mice received an intravenous injection of 250 µL of phosphate buffered saline (PBS) containing 20 µCi of ^{3}H-thymidine via the lateral tail vein. Five hours later, the mice were sacrificed and the draining auricular lymph nodes excised and a single cell suspension of lymph node cells was prepared for each mouse. A mean disintegration per minute (dpm) value ± SD (standard deviation) was calculated for each experimental group. In addition, a Stimulation Index (SI) was calculated for each mouse using the following equation:

SI= dpm of individual mouse / average dpm of the vehicle control mice

Table 8.3. Summary of lymph node proliferation data

Treatment group	DPM (mean ± SD)	SI (mean ± SD)
Vehicle (DMF)	2015 ±749	1.0 ± 0.4
HCA (30% v/v)	5970 ± 1909*	3.0 ± 0.9

2% HBCD	1744 ± 181	0.9 ± 0.1
20% HBCD	1670 ± 155	0.8 ± 0.1
50% HBCD	1946 ± 664	1.0 ± 0.3

DPM=disintegration per minute

* represents statistical difference from control mean at $p<0.05$ using Dunnet's T-test

Chemicals that elicit an SI of ≥3 in the LLNA are considered positive for dermal sensitisation potential. Clinical observations were carried out throughout the study with the following parameters being evaluated: skin, fur, mucous mambranes, respiration, nervous system function (including tremors and convulsions) animal behaviour, moribundity, mortality and the availability of feed and water. Results of the LLNA are presented in Table 8.3.

The SI values were consistently around 1.0 at all doses tested. The conclusion of the LLNA was that HBCD was not a skin sensitiser.

According to the EU RAR (2008), different results obtained from studies using the EU-marketed HBCD and the Japan-marketed HBCD (Nakamura et al. 1994) could be due to the difference in composition of the test material used (e.g. additives, impurities). Also, the use of acetone as a vehicle for HBCD could have promoted its penetration on shaved skin in the challenge phase of the Japanese studies versus the use of the suspended substance in corn oil on clipped skin in the US study (Wolhiser & Anderson 2003). However, as the LLNA also involved HBCD completely dissolved in DMF, the most likely explanation of the different results was the presence of different impurities in the EU- and Japan-marketed HBCD.

Respiratory tract

No data are available to assess the potential for respiratory sensitisation (EU RAR 2008).

8.2.4 Repeat dose toxicity

28-day studies

In a 28 d study (Chengelis, 1997), commercial HBCD in corn oil was administered by gastric intubation to Sprague-Dawley Crl:CD BR rats as a single daily dose for 28 consecutive days according to OECD TG 407 and in compliance with US EPA GLP. A dose volume of 5 mL/kg was used for all dosage levels. The doses used were 125 (low), 350 (mid) or 1000 (high) mg/kg bw/d. The test groups consisted of 12 animals (6/sex/group) in the 125 and 350 mg/kg bw/d, and 24 animals (12/sex/group) in the 1000 mg/kg bw/d group. A concurrent control group comprising 24 animals (12/sex/group) received 5 mL/kg bw of the vehicle, corn oil, for 28 consecutive days. The composition of HBCD was α isomer (6.3%), β isomer (9.1%) and γ isomer (76.9%). The animals were observed twice daily for mortality and moribundity. Clinical observations were conducted on all animals at the time of dosing and approximately 1 to 2 h following dosing. At the end of the dosing period, 6 animals/sex/group were sacrificed and necropsied. The remaining 6 animals/sex in the control and 1000 mg/kg bw/d groups remained untreated for a 14 d recovery period. During the 14 d recovery period, clinical observations were performed daily.

Clinical signs observed during the study were non-specific and not related to the test substance. Bodyweight gain and food consumption of treated animals were not affected by treatment. No statistically significant differences in bodyweight between control and treated animals were detected with the exception of an increase in mean bodyweight in female rats in the 350 mg/kg bw/d group in the treatment stage, and a decrease in mean male bodyweight gain in the 1000 mg/kg d group in the recovery stage.

Following the 28 d treatment, a statistically significant increase in the relative liver weights were observed at the mid and high doses in male rats and at all doses in females rats (up to 40% increase at the high dose), compared to control animals. No related histopathological and serum chemistry changes were observed, which indicated that the liver weight changes were an adaptive response characterised by induction of the microsomal enzymes. At sacrifice after the recovery period, the relative liver weight in males had normalised to control levels. The relative liver weight in females had decreased but was still significantly elevated (+17%) in the high dose group in comparison to control rats.

A slightly increased colloid loss in the thyroids of high-dose males when compared to controls was noted. This effect was not seen in females. Histopathological examination of the thyroid did not reveal any significant lesions. The thyroid effects of HBCD are presented in Section 8.4.2. In conclusion, on the basis that liver weight changes were still significantly high in the high dose females after the recovery period, the NOAEL was considered to be 350 mg/kg bw/d.

In another 28 d study (Zeller & Kirsch 1969), HBCD (Hexabromid S) was administered to Sprague-Dawley rats in groups of 10/sex/group at doses of 0 (control), 1% (Group 2), 2.5% (Group 3) and 5% (Group 4) of the diet for a period of 4 weeks. The doses calculated from the actual bodyweights and food consumption in the study were 0, 940, 2410 and 4820 mg/kg bw/d.

At the 1% dose level (Group 2), no clinical signs related to treatment were observed. At the 2.5% and 5.0% dose levels (Groups 3 and 4) the rats were in poor condition, took less food and therefore had inhibited growth. Bodyweights were comparable to the controls in the 1% and 2.5% dose levels (Groups 2 and 3).

No significant changes were noted in blood or urine parameters. Liver weights (absolute and relative to bodyweight) were increased at all dose levels when compared to control levels, but no histopathological changes were noted. Microfollicular hyperplasia and increased activity of the epithelium in the animals' thyroids were noted in Group 2 animals. The changes were more marked in Group 3, and in Group 4 there was very marked hyperplastic thyroid tissue with adenomatous proliferation and epithelial hyperactivity. These changes were ascribed to increased activity of the organ and cells. There were no changes in any other organ that were related to HBCD intake. This study did not comply with OECD guidelines and, as the liver weight increases were noted at all dose levels, a NOAEL could not be determined based on these data.

In a 28 d oral study in rats, carried out according to the OECD TG 407 (Van der Ven et al. 2006), Wistar rats of both sexes, aged 11 weeks, were dosed with HBCD (a mixture of 3 enantiomers – HBCD-α, -β, and -γ – with a proportion 10.28%, 8.72%, and 81.01%, respectively) dissolved in corn oil. Target dosing was 0, 0.3, 1, 3, 10, 30, 100, and 200 mg/kg bw/d (5 rats/sex/dose group); the highest dose depended on maximum HBCD solubility in corn oil.

Animals were inspected daily for general condition and clinical abnormalities and their bodyweight and feed consumption was recorded. At the end of the exposure period, animals were necropsied in the course of 5 d, resulting in a total of 28 to 33 d of exposure. Body organs were collected and weighed directly after dissection or after fixation (thyroid, pituitary) to reduce mechanical damage. Sperm was collected from the cauda epididymis for direct analysis of motility and morphology. Microscopic observations (for histopathology) were done by comparison of control and exposed samples. Clinical chemistry, thyroid hormone (TH) analysis, apolar retinoid analyses and bone analyses were carried out for all groups.

The α-, β-, γ-HBCD diastereomers were determined with LC-MS/MS using electrospray ionisation. Identification of HBCD was based on the retention times of the ^{13}C-labeled compounds and the bromine clusters of the adduct ion m/z 677 and fragment ion m/z 644. Measurement of the β-HBCD enantiomer in a selected number of tissue samples showed that this isomer only comprised a minor fraction of the total HBCD (< 1.5%; data not shown); measurements in the full experimental set were therefore restricted to the HBCD α and γ enantiomers.

Statistical analysis, based on external dosing (mg/kg bw) was conducted using a nested family of purely descriptive (exponential) models with the PROAST software. From the best curve fit, indicated by significance at the 5% level, a critical effect dose (CED), also referred to as benchmark dose (BMD), was calculated at a predefined critical effect size (CES) of 10% or 20%. CES is defined as the threshold adverse effect level, determined by expert judgment, for each parameter based on knowledge of the pathophysiology of each effect, including irreversibility or adverse follow-up effects. In practice, a CES of 10% was defined as the default, considering that this effect size will cover the hazard for the most sensitive subjects in a population. For liver weight and immune parameters, a CES of 20% was defined. The analysis was completed with the calculation of a 95% confidence interval (2-sided), thus enabling the calculation of a 5% lower confidence bound of the CED estimate. This value may be considered as a benchmark dose at the lower confidence bound (BMDL) for continuous data.

Individual dose groups were not compared due to relatively few replicates per dose group. Some parameters, which were targeted at discerning an overall difference rather than a dose-response, were analysed using conventional statistical tests, such as Fisher exact test, two-way ANOVA and a Student t-test.

There were no effects on body growth during the exposure period. Analysis of α-HBCD and γ-HBCD in the liver showed a dose-dependent increase with a plateau at the 3 highest doses. HBCD concentrations in the liver were higher in females than in males over the entire dose range (on average 5.2 times). The average ratio γ:α, which was approximately 8:1 in the administered technical mix, appeared to be 2.9 in females and 1.9 in males and decreased with dose in both sexes; at low doses, there was a higher contribution of the -γ enantiomer, while at higher doses the ratio equalized or reversed. At doses of 100 mg/kg bw/d and above, the relative concentration of α increased much further compared to γ. The authors explain this as a result of either reduced absorption or increased metabolism of γ at high doses. The latter is possibly explained by induction of liver enzymes that specifically metabolise the γ isomer.

In female rats, there was a significant dose-dependent increase in liver weight and induction of the liver enzyme T4-uridine-diphosphate glucoronyl transferase (T4-UDPGT). There was also a significant dose-dependent increase in pituitary and thyroid weights in females accompanied by a decrease in T4. Thyroid follicles were smaller and depleted, and had activated hypertrophied epithelium in females. Cell height and nuclear size were most sensitive, with initiation of the effect (a progression from slight to moderate activation, i.e. size) at intermediate doses, whereas effects on follicle size and cytoplasmic vacuolization only appeared at the high end of the dose range. Comparison of top dose animals with control animals showed a significantly increased ratio between high- and low-intensity immunostaining thyrotropic cells in females, consistent with the increase of pituitary weight.

Table 8.4. Effect of HBCD on some organ weights in rats

Dose*	Pituitary (mg)		Thyroid (mg)		Liver (g)	
	M	F	M	F	M	F
0	10 ± 2	5 ± 1	27 ± 4.8	17 ± 2	13.9 ± .7	9.7 ± 1
0.3	6 ± 2	12 ± 1	23 ± 5.0	18 ±1	17.1 ± 3	8.9 ± 1.1
1	14 ± 2	11 ± 3	26 ± 5.4	22 ±4	16.2 ± 3	8.6 ± 1.3
3	8 ± 2.	13 ± 2	28 ± 5.6	15 ± 4	15 ± 1.6	9.5 ± 0.4
10	12 ± 4	11 ± 3	27 ± 8.6	18 ± 3	17.7 ± 2	8.9 ± 0.6
30	10 ± 2	8 ± 2	27 ± 4.3	35 ± 17	15.7 ± .5	11 ± 1.0
100	8 ± 4	13 ± 1	24 ± 3.5	27 ± 7	16.4 ± 2	13 ± 0.5
200	11 ± 3	13 ± 2	25 ± 5.9	26 ±3	16.4 ± 3	12 ± 0.6
BMDL	NSE	29.9	NSE	1.6	NSE	22.9

Figures are averages±SD; *mg/kg bw/d HBCD; M=Male rats, F=Female rats; NSE = no significant effect.

In male rats, a decrease in thymus weight was noted at 200 mg/kg bw when compared to control animals. There were no significant changes in pituitary, thyroid and liver weights.

Female rats had decreased concentrations of alkaline phosphatase and glucose and increased cholesterol and total protein/albumin. Males had decreased cholesterol, increased total protein/albumin and decreased glucose, all in a significant dose-dependent way. Measurement of thyroid hormones showed no changes in T3 levels but a dose-dependent decrease in total thyroxine (T4) in females only. In both male and female liver, a dose-dependent increase of T4-UDPGT activity was found. The hypothyroid-like effects seen in these rats were suggested to be due to decreased plasma T4 levels. The initiating event is probably activation of liver enzymes (induction of T4-UDPGT), resulting in increased liver weight at 22.9 mg/kg bw/d and increased TH turnover (decreased T4 at 55.5 mg/kg bw/d) and a feedback reaction in the pituitary and the thyroid gland as evidenced by increase in their weights and increased immunostaining intensity of thyroid stimulating hormone (TSH).

Mineral density was higher in the trabecular bone at femur and tibia metaphysis in female rats. There were no changes in any of the bone parameters in male rats. The BMDL for liver weight increase was 22.9 mg/kg bw/d, using a 20% weight increase as the critical effect level. The probable reason for such an increase is the hepatic enzyme induction. Data from this study indicate that liver weight increases in female rats were apparent from 30 mg/kg bw/d onwards, giving a low observed adverse effect level (LOAEL) of 30 mg/kg bw/d and a NOAEL of 10 mg/kg bw/d.

90-day studies

In an early 90 d study (Zeller & Kirsch 1970), HBCD (Hexabromid S) was administered to groups of 40 Sprague-Dawley rats (20/sex/group) at doses of 0%, 0.16%, 0.32%, 0.64% or 1.28% in the diet. The calculated doses based on the actual bodyweights and food consumption were equivalent to 0, 120, 470 and 950 mg/kg bw/d. A concurrent control group was fed rodent diet. After the exposure period, 10 extra rats per sex from the control group and the highest dose group were kept for 42 d on control food.

There were no clinical signs of toxicity and no changes in bodyweight and clinical chemistry in rats treated with HBCD up to 950 mg/kg bw/d.

Liver weights of both males and females in each of the experimental groups were greater than controls. Liver:bodyweight ratios of males and females increased in a dose-related manner consistent with the level of HBCD in the diet. Sporadic differences were noted in kidney:body, and heart:bodyweight ratios. The liver:bodyweight ratios remained high, but there was no difference between the means of the liver:heart weight ratios. Increased incidence of hepatic lipoid phanerosis (fatty accumulation) was observed in the livers of many animals at all doses, with a general tendency to increase with the level of HBCD in the diet. In the absence of detectable clinico-chemical disturbances or histological changes in the vital organs, it was concluded that the changes which were largely reversible when the administration of hexabromid S was stopped were due to a transient increase in liver activity. The study was not conducted according to OECD guidelines; however, based on the limited observations in this study, the NOAEL was determined to be 950 mg/kg bw/d.

In another 90 d oral toxicity study, conducted according to GLP and OECD TG 408, HBCD as a suspension in corn oil was administered to 4 groups of Sprague Dawley rats, 15/sex/group by oral gavage, once daily at 0, 100 (low), 300 (mid) or 1000 mg (high) HBCD/kg bw/d, 7 d per week for 90 d (Chengelis, 2001). In addition to the main group, two other groups of 20 animals/sex/group (satellite groups) were treated concurrently at doses of 0 or 1000 mg/kg bw/d for up to 90 d. The animals in this group were used for the collection of tissues for special analyses. The test material which was a mixture of 3 commercial lots contained 90% pure HBCD. Bodyweights and food consumption were measured weekly and blood and urine examinations were conducted during the observation at weeks 3 to 13. Functional Observational Battery (FOB) and Locomotor Activity (MA) evaluations were performed prior to initiation of dosing, in week 13 and during the recovery period. Vaginal cytology was conducted on study days 69 and 90. In the satellite group, 2 animals/sex/group were euthanised on study days 2, 6, 9, 13, 20, 27, 55, 89, 104 and 118, and blood and body fat were collected and analysed for HBCD content.

There were no effects on food consumption, bodyweight, haematology parameters, oestrous cycle of females or the male reproductive system. There were no changes in FOB and MA. Statistically significant clinical chemistry changes at week 13 included an increase in albumin (at all doses in males), total protein (at all doses for females and 1000 mg/kg bw/d for males), globulin (300 and 1000 mg/kg bw/d for females) and chloride (all doses for both sexes). In addition, increased γ-glutamyltransferase levels were noted in the 1000 mg/kg bw/d group. The changes were not of sufficient magnitude to be adverse, as they occurred in otherwise clinically-normal animals and tended to be within or close to historical or control values.

There were dose-dependent statistically significant increased relative liver weights in both sexes in all dose groups (18% to 44% in males, and 24% to 48% in females). Minimal hepatocellular vacuolisation was observed in both sexes at all dose groups. Some females in the mid and high dose groups exhibited mild to moderate hepatocellular vacuolisation. Minimal to mild hepatocellular hypertrophy was observed in high-dose females. In the highest dose group (1000 mg/kg bw/d), effects on the relative liver weight remained after the 28 d recovery period, although they were statistically significant only in the males.

T4 levels were decreased at study week 13 compared to controls in males at all doses, and at 300 mg/kg bw/d and 1000 mg/kg bw/d in females. There were no corresponding increases in T3 and TSH levels. Histological changes in the thyroid consisted of a slight increase in the incidence of minimal follicular cell hypertrophy in the high-dose males and minimal or mild hypertrophy in the high-dose females.

The mean prostate weight was increased in the 1000 mg/kg bw/d group. HBCD was detected in the adipose tissue of male and female rats treated with 1000 mg/kg bw/d for up to 90 d. Isomer-specific analysis showed that the relative isomer concentrations in adipose tissue at all time points were α>>γ>β as compared to the test article in which the γ isomer was the highest followed by α and then the β isomer. Steady state levels were achieved by study day 27. Levels in male and female rats were similar at all time points and declined during the recovery period.

In conclusion, all HBCD-related changes at 100 and 300 mg/kg bw/d were mild and reversible and probably secondary to hepatic enzyme induction, which is generally an adaptive change. Similarly, it was not apparent that the minimal irreversible changes noted in the thyroid were a direct effect of treatment. Follicular cell hypertrophy is the normal physiological response to reduced serum T4 levels (which was noted here), and is the typical adaptive response of a normal functioning organism acting to maintain normal serum T4 levels. The increased prostatic weight was not considered significant as the increase did not persist till the recovery period, there were no correlating histological changes and no changes in seminal quality. The effects at 1000 mg/kg bw/d were partially reversible, although the relative liver weight changes were still apparent at 28 d post-treatment. The study report concluded a NOAEL of 1000 mg/kg bw/d, with the argument that the effects on the liver were not very serious and seemingly slowly reversible. However, based on significant increases in relative liver weights in both male and female rats (24% in female rats), a LOAEL of 100 mg/kg bw was identified in the study.

In a lifetime bioassay (Kurokawa et al. 1984), 200 B6C3F1 mice of each sex were divided into 4 dose groups and exposed to HBCD in the diet for 18 months. The doses of 0 (control group), 199, 1000 or 10 000 ppm were equivalent to 0, 13, 130 and 1300 mg/kg bw/d respectively. The HBCD used was a fine white powder, soluble in acetone and xylene, slightly soluble in benzene and olive oil and insoluble in water. Daily observations were made on the general condition of the mice, and bodyweight and diet consumption were measured once every week during the first 3 months and then once every month. The mice were sacrificed after the administration period, and the following organs were collected for histopathological examination: brain, heart, lung, liver, kidneys, spleen, male and female reproductive organs, pituitary, thyroid, submandibular gland, thymus, adrenals, oesophagus, stomach, small intestine, pancreas, rectum, urinary bladder, mesenteric lymph nodes, sternum and femur.

There was no difference in mortality between the groups. The bodyweight was lower in the exposed groups, but no apparent signs of toxicity were reported. Overall, the effects noted in the various organs, including the liver, lung, kidneys and uterus, did not exhibit a dose-response effect. The changes in the liver were difficult to interpret due to lack of description of severity and the absence of a clear-cut dose response effect. This may have been due to the low absorption of HBCD particles, especially at the highest concentration of HBCD in the feed. The cancer incidence results from this study are presented in Section 8.2.6.

This study was not conducted according to OECD guidelines, and details of the methodology used were not provided. Therefore, a NOAEL was not established from the study.

No dermal or inhalation repeat-dose studies were available.

Overall summary of repeat dose effects of HBCD

Repeat dose inhalation or dermal studies were not available. The oral repeated dose toxicity studies (28 d and 90 d) demonstrated a consistent increase in liver weight in rats exposed to HBCD. The increased liver weight was accompanied by minimal pathological findings in the 90 d study. No histopathological changes were observed in 28 d studies in rats. The increases in liver weights were reversible except at very high doses in the 90 d study. The increase in liver weight could be an adaptive change characterised by liver enzyme induction. However, a >20% increase in liver weight in the 28 d Van der Ven et al. (2006) study and 40% increase in liver weight in female rats in the 90 d study are quite significant. Enzyme induction is known to occur in humans too. This effect is therefore relevant for the risk characterisation. A NOAEL of 10 mg/kg bw/d was established based on increase in liver weights at the next higher dose (30 mg/kg bw/d). Increased pituitary and thyroid weights were also observed in the 28 d and 90 d studies, with histopathological changes in the thyroid.

8.2.5 Genotoxicity

In-vitro assays

Bacterial reverse mutation assay

A mutagenicity test was carried out for Pyroguard SR-103 (pure HBCD in DMSO) using 6 *Salmonella typhimurium* strains (Ogaswara & Hanafusa 1993).

HBCD was found to be non-mutagenic at up to 10 000 µg/plate in 6 *S. typhimurium* strains (TA92, TA1535, TA100, TA94, TA1537 and TA98) in both the presence and absence of exogenous metabolic activation (NADPH-induced rat liver S9 fraction). The number of colonies were counted after incubation at 37 ºC for 2 d. Two plates were used for each concentration. Although the study details provided were minimal, it can be concluded that Pyroguard SR-103 (HBCD) was not mutagenic to *S. typhimurium*.

In another mutagenicity study 5 strains of *Salmonella typhimurium* (TA1535, TA1537, TA1538, TA98 and TA100) were tested in the presence and absence of a metabolic activation system (from Aroclor-induced rat liver) at doses of 1, 10, 540, 100, 500, 1000 and 5000 µg/plate. Results indicated that HBCD was neither mutagenic nor cytotoxic in the studies (Simmon* et al. 1976).

Chromosomal aberration test

A GLP compliant structural chromosome aberration study (OECD TG 473) was conducted on HBCD using human peripheral blood lymphocytes in the absence and presence of metabolic activation (Aroclor 1254-induced rat liver S9 fraction) (Gudi & Schadly 1996). Concentration ranges up to 2500 µg/mL were used. Treatment was for 4 h, with fixation at 20 or 44 h. The assay was performed in 2 phases. The first phase, the initial chromosome aberration assay, was conducted to establish the dose range for testing and to evaluate the clastogenic potential of the test article. The second phase, the independent repeat chromosome aberration assay, was performed to confirm the test system response to the test article seen in the initial test. HBCD was concluded to be negative for the induction of structural and numerical chromosome aberration in human peripheral blood lymphocytes.

Other assays

The effects of HBCD on somatic recombination at an endogenous locus in mammalian cells were studied in a non-standard assay using 2 Chinese hamster cell lines containing duplication mutations in the *hrpt* gene (Helleday et al. 1999). Other brominated flame retardants and several other well-known environmental pollutants were also tested. The endpoints used were the in-vitro Sp5/V79 (Sp5) and the in-vitro SDP8 recombination assays.

The protocol for treatment was identical for the 2 cell lines, with treatments being conducted for 24 h in Hank's minimal essential medium (HMEM). All chemicals were dissolved in DMSO prior to treatment and added to the medium at a final concentration of 0.2%. Camptothecin (100 nM) was used as a positive control. The HBCD doses used were 0 (control), 3, 6, 10, 15 and 20 µg/mL respectively. Results showed that the recombination frequency was significantly elevated 2.2-fold in the Sp5 assay and 1.9-fold in SDP8 cells. Both of these effects demonstrated statistically significant dose dependence. However, in comparison with a clearly recombinogenic agent like Cr (VI), the effect was small, and its biological significance with respect to the in vivo situation is not clear.

In-vivo assays

Micronucleus assay

HBCD was tested for clastogenicity and for the ability to induce spindle poison effects in NMRI mice using the micronucleus test method, as specified in OECD TG 474 and according to GLP (Engelhardt & Hoffman, 2000). The micronucleus test enables the indirect detection of a chromosome-damaging (clastogenic) effect or damage of the mitotic apparatus (spindle poison effect).

In the test, 90% pure HBCD was dissolved in DMSO and administered twice intraperitoneally with a 24 h interval to male mice at dose levels of 500 (low), 1000 (intermediate) or 2000 (high) mg/kg bw in a volume of 4 mL/kg bw. Cyclophosphamide and vincristine were administered as positive controls for clastogenic effects and spindle poison effects respectively. DMSO was used as a solvent control. Five animals were used per test dose group, 1 in vehicle control and 2 in positive control groups. The administration of HBCD (first administration) led to evident clinical signs of toxicity such as the squatting posture. The control animals did not show any signs of toxicity. Twenty-four hours after the second administration the animals were sacrificed, and the bone marrow of the 2 femurs was stained and 2000 polychromatic erythrocytes were evaluated per animal and checked for micronuclei. Results showed no biologically relevant significant differences in the frequency of erythrocytes containing micronuclei between the vehicle control and the low-, intermediate- and high-dose groups. Therefore, under the experimental conditions HBCD had no chromosome-damaging (clastogenic) effect and did not lead to any impairment of chromosome distribution in the course of mitosis.

8.2.6 Carcinogenicity

Oral

In a chronic study (Kurokawa et al., 1984) (see Section 8.2.4), 200 B6C3F1 mice of each sex were divided into 4 dose groups and exposed to HBCD at doses of 199, 1000 or 10 000 ppm for 18 months (equivalent to 13 (low), 130 (mid) and 1300 (high) mg/kg respectively). The control group contained 100 animals (50/sex/group).

There was no difference in mortality between the groups. Macroscopic examination showed liver nodules, mainly in males, at all doses (low dose, 21/50; mid dose, 30/50; high dose, 24/50; and controls, 12/50), although no dose-response relationship was noted. On histopathological examination of the liver, hepatocellular carcinomas were noted mainly in males (low dose, 17/50; mid dose, 25/50; high dose, 13/50; and controls, 12/50). There was a small treatment-related increase in hepatocellular carcinomas, but no dose-response effect was seen. The incidences at low and mid doses were higher than at the highest dose, and the incidence at the highest dose was similar to that in controls. The pathology report was not available.

Histopathological examination of the lungs revealed the presence of adenomas in both males and females, with males showing a higher incidence (low dose, 9/50; mid dose, 8/50; high dose, 5/50; and controls, 5/50) and females showing lower incidence (low dose, 5/50; mid dose, 3/50; high dose, 2/50; and controls, 1/50) and no dose dependence. No dose-response effects were noted. This study was not conducted according to OECD guidelines, and details of the methodology used and the histopathological findings were not available.

In conclusion, there was no evidence of HBCD-related carcinogenicity in this study.

Dermal

No dermal carcinogenicity studies are available.

8.2.7 Reproductive toxicity

Fertility

A 2-generation reproductive toxicity study was conducted in Crl:CD(SD)IGS BR rats (Ema et al., 2008). The study was performed according to OECD TG 416 and in accordance with the principles of good laboratory practice.

Twenty-four F0 rats (5-week old)/sex/group were fed a diet containing HBCD at 0, 150, 1500 or 15 000 ppm for 10 weeks prior to mating period. Administration of HBCD was continued throughout the mating, gestation and lactation periods. The mean daily intakes of HBCD during the whole period were 10.2, 101 and 1008 mg/kg bw in F0 males and 14, 141 and 1363 mg/kg bw in F0 females. Each female was mated with a single male of the same dosage group (mating periods were typically 3 weeks). The day of successful mating (indicated by vaginal smear) was designated as day 0 of pregnancy.

Twenty-four male and 24 female F1 weanlings in each group were selected as F1 parents on PNDs 21 to 25 (designated 0 week of dosing for the F1 generation). F1 selected rats were administered HBCD in the diet. Administration of HBCD in the diet continued throughout mating, gestation and lactation periods. Females were allowed to deliver spontaneously and nurse their pups unil PND 21 (the day of weaning). On PND 26, F1 weanlings, in excess to those selected as F1 parents, and all F2 weanlings were necropsied.

Litter data (total litter size and the number of live and dead pups) were collected and development and behavioral landmarks of F1 and F2 pups (anogenital distances, spontaneous locomotor activity) were recorded.

Bodyweights and food consumption were recorded weekly. Parental rats were necropsied and organ weights were recorded and hispathological evaluations of F0 and F1 adults were performed. Haematological examinations were performed for 10 males and 10 females of F0 and F1 rats randomly selected from each group. Sperm parameters were also determined for all F0 and F1 male adults on the day of scheduled sacrifice.

General toxicity in F0 and F1 rats

No significant difference in the incidence of clinical signs of toxicity in either male or female F0 and F1 rats was seen between control and HBCD-treated groups during the test period. Bodyweights were significantly higher in F0 rats at higher doses and significantly lower than the controls in F1 rats at the higher dose.

Increased absolute and relative weights of the liver were noted at the top dose regardless of sex, age and generation (F0 and F1). In F0 males, there was a significantly decreased relative weight of brain at 1500 ppm and of the seminal vesicles at the top 2 doses. The F0 females had significantly increased absolute weight of the thyroid and adrenals at 15 000 ppm when compared to controls.

In F1 males there were significant increases in testis weight at 150 ppm only. Changes in absolute and relative weights of other organs, such as kidney, brain, testis and pituitary, did not show any dose response and sometimes occurred at only 1 dose level.

There were no significant changes in T3 levels in F0 and F1 rats of both sexes. Lower levels of T4 compared to controls were observed at 15 000 ppm in F0 males and females. Statistically significant increases in TSH levels were found in F0 and F1 females at 1500 ppm and above (also at 150 ppm in F0 females).

There were no compound-related gross lesions or remarkable microscopic alterations in tissues and organs, except for the thyroid in the adult rats. Decreased size of follicles in the thyroid was found in F0 and F1 adults at 1500 ppm HBCD and higher doses and in F1 females at 150 ppm and higher doses. Hypertrophy of the follicular cells in the thyroid was also observed in F0 males and females at 1500 ppm and higher. There were no compound-related gross lesions and histopathological changes in thyroid in male and female F1 and F2 pups and weanlings.

Effect on fertility

F0 rats

No significant differences between control and HBCD-treated rats were seen in copulation index, gestation index, pre-coital interval, number of implantations, delivery index or number of F1 pups delivered. There were only slight reductions in the fertility index (copulation abiliy and impregnation ability) in F0 and F1 rats but not significantly different from those in the control group.

A significantly lower number of epididymal sperm at 150 ppm and higher mean amplitude of lateral head displacement at 15 000 ppm was found in males compared to controls.

Significantly longer gestation length and lower sex ratio of live pups were noted at 1500 ppm HBCD. However no effects on sex-hormone-dependent events, such as weight of reproductive organs and estrous cyclicity, were apparent. At the highest dose (15 000 ppm), the proportion of pregnant females to total number of mated females was significantly lower than that of controls.

No significant differences in serum testosterone, estradiol, progesterone and LH levels were noted in adults of both sexes between control and HBCD-treated groups. Compound-related gross lesions or microscopic alterations were not observed in reproductive organs in male or female F0 and F1 rats.

F1 rats

In F1 males there were no significant changes in the sperm counts, the percentage of motile sperm, sperm swimming speed and pattern and the percentage of morphologically abnormal sperm. No significant effect of HBCD was seen on copulation and fertility indexes. The absolute and relative weights of testes were higher at all dose levels.

In female rats, no significant effect was found on the estrous cycles. There were no significant differences in the copulation index, fertility index, gestation index, pre-coital interval, gestation lengths, number of implantations, delivery index, or number of F2 pups delivered between the control and HBCD-treated groups.

The absolute and relative ovary weights were higher at 150 ppm but not different from those of controls at higher doses, although higher relative weight of ovaries was observed at the highest dose (15 000 ppm).

The number of primordial follicles in the ovary of F1 females was significantly decreased at 1500 and 15 000 ppm, compared to controls (measured only in F1 females). Although these findings suggest that HBCD has the potential to affect fertility, no adverse effects on other fertility parameters in F1 dams, or on the numbers of implantations or F2 pups delivered, were noted in the present study. The reduction in the number of follicles was the same at 1500 and 15 000 ppm HBCD and no reduction was observed at 150 ppm. Moreover, the reduced mean values observed in treated rats were within historical control values from the same laboratory and species of rats. No compound-related gross lesions and microscopic alterations could be found in any organs except for the thyroid, where a decreased size of follicles could be seen. The authors suggest that a continuous breeding study of HBCD may be needed to clarify the reproductive toxicity of HBCD, especially the adverse effects of HBCD on the reproductive life span.

No significant differences in serum testosterone, estradiol, progesterone and LH levels were noted in F1 adults of both sexes between control and HBCD-treated groups. HBCD did not affect sex-hormone-dependent events, such as weight of reproductive organs and estrous cyclicity. No compound-related gross lesions or microscopic alterations were observed in reproductive organs in male and female F1 rats.

A NOAEL for effect on fertility could not be established from this study due to lack of a clear dose-dependent effect on fertility-related parameters.

Van der Ven et al. (2009) determined the endocrine effects of HBCD in a 1-generation reproduction study in Wistar rats. The experimental protocol of the study was based on the OECD TG 415. Parental (P-generation) animals (10 animals/sex) were fed diets containing HBCD formulated to attain doses of 0, 0.1, 0.3, 1,3, 10, 30 or 100 mg/kg bw/d HBCD (calculated based on results of earlier studies). Exposure started 70 d or 14 d prior to mating for male and female rats, respectively. The day of observed presence of sperm or a vaginal plug was recorded as day 0 of pregnancy.

P-generation animals were euthanised after mating (males) or after weaning (females), with recording of the number of uterus implantation sites in the latter. During lactation, pups were exposed to HBCD via the milk, and also had access to the feed of the dam. At weaning (PND21), 2 male and 2 female F1 animals were euthenised for weighing of reproductive organs. The remaining F1 animals were maintained under the same dosing regime as their parents. Near the end of the dosing period, animals were assigned to groups for either neurobehavioural tests, immunisation assay or for necropsy to study effects of dietary HBCD on body organs.

Haematology, clinical chemistry, thyroid hormone analyses, apolar retinoid analyses, bone marrow analyses and tests for steroid hormone synthesis and immunological parameters were carried out in these animals.

No significant difference was seen between control and HBCD-treated groups in the incidence of clinical signs of toxicity in either male or female F0 (parental) and F1 rats during the test period. Analyses of α- and γ-HBCD in the liver of F1 animals showed a dose-dependent increase of these stereoisomers in treated rats. The HBCD concentrations were higher in the liver of F1 females compared to males over the entire range of doses. There were no dose-response effects on endpoints of reproduction (mating success, gestation duration, number of implantation sites and litter size). Testes weights were decreased at lower doses in F1 animals, while kidney, thymus, adrenals, prostate, heart and brain weights were lower only at higher doses. However, these changes were not considered to be of toxicological significance, as there were no remarkable histopathological changes in any of these organs. There were no effects on thyroid hormones in either sex or any histopathological changes observed in thyroid glands.

In addition to the above reproductive toxicity studies, reproductive effects from the repeat dose studies discussed in Section 8.2.4 are presented below.

In a 28 d repeat-dose study (Zeller & Kirsch, 1969), Sprague-Dawley rats (40/sex/group) divided into 1 control group and 4 test groups were administered orally 1.0%, 2.5% and 5% equivalent to 940 (low dose), 2400 (mid dose) and 4700 (high dose) mg/kg bw/d HBCD. Hypoplastic inner genitalia were noted at autopsy in the high-dose group. Inhibited oogenesis and a low number of the follicles and ripened follicles in the ovaries were reported in females at the highest dose. The testes and epididymes of the animals in the high-dose group showed normal differentiation of the inner sexual organs and undisturbed spermiohistogenesis, although the latter were small.

In a 90 d oral (gavage) study conducted in rats (Chengelis, 2001), the mean prostate weight was increased in the 1000 mg/kg bw/d group. However, this was not considered to be of toxicological significance, as the increase did not persist through the recovery period. There were no correlated histologic findings and no change in seminal quality.

In an 18-month oral study conducted in mice fed HBCD at 100, 10 000 and 50 000 ppm, equivalent to 13, 130 and 1300 mg/kg bw/d, swelling of the uterus was noted in all 3 groups (27, 24 and 3 cases, respectively), and also in the control group (31 cases). Histopathological changes in the uterus were also noted in all groups including the control group (Kurokawa et al., 1984).

Developmental toxicity

A 7 d dose range finding study and a combined teratogenicity-developmental study were conducted in rats (Murai et al., 1985). In the range-finding study, 5 rats/dose group were given 0, 300, 1300 or 10 000 mg/kg bw/d. Doses as high as 10 000 mg/kg bw/d produced no evidence of toxicity. A statistically significant increase in liver weight was noted in groups receiving ≥1000 mg/kg bw/d. Doses for the combined teratogenicity–developmental study were based on this increase in liver weight.

In the teratogenicity–developmental study (Murai et al., 1985), pregnant rats were fed diets containing 0%, 0.01%, 0.1% or 1% HBCD, approximately equivalent to 0, 7.5, 75, and 750 mg/kg bw/d, respectively, on days 0 to 20 of gestation. Rats were observed daily and bodyweight and food consumption were measured. On day 20 of gestation, 14 rats from each group were sacrificed and their foetuses examined for toxicity and teratogenicity. Approximately 150 foetuses per dose level were examined for evidence of teratogenicity. All foetuses from all litters were examined for external anomalies. Approximately two-thirds of the foetuses per dam were examined for skeletal abnormalities; the remaining foetuses were examined for visceral damage The remaining 6 dams delivered naturally, and the pups were monitored through weaning.

No adverse effects were detected in the dams of any treatment group with respect to food consumption, maternal weight gain or gross appearance of internal organs. Bodyweight changes were comparable in all groups. The mean liver weight in the 1% (750 mg/kg bw/d HBCD) group was higher (statistically) than the control mean.

There was no treatment-related adverse effect of treatment on the number of corpora lutea, implants, resorptions, live fetuses, sex ratio, or body or placental weights. No external, skeletal or visceral malformations were detected. There were no significant differences between the control and treated groups in the number of implantations, live or dead newborns or live newborn parturition index. The weaning and survival index was comparable in the control and treated groups. Normal development was seen in neonates up to 6 weeks of age.

No reproductive or developmental effects were noted in rats treated with HBCD at doses of up to 1% (750 mg/kg bw/d) administered from days 0 to 20 of gestation. Based on the above findings HBCD did not demonstrate any teratogenic potential in this study. The developmental toxicity NOAEL was 750 mg/kg bw/d, the highest dose tested, and the maternal toxicity NOAEL was 75 mg/kg bw/d, with a liver weight increase of 13% at the next higher dose.

In a developmental toxicity study (Stump, 1999), groups of 25 female Charles River CD rats were administered HBCD at doses of 0, 250, 500 or 1000 mg/kg bw/d at a dose volume of 5 mL/kg in corn oil, once daily from gestation days 6 through to 19. The test substance was administered orally by gastric intubation. Clinical observations, bodyweights and food consumption were recorded.

On day 20, all females were subjected to laparohysterectomy. The uteri and ovaries were checked and the number of foetuses, early and late resorptions, total implantations and number of corpora lutea were recorded. Mean gravid uterine weights and net bodyweight changes were calculated for each group. The foetuses were weighed and checked for external soft tissue and skeletal malformations as well as variations. There were no mortalities during the gestation period. Bodyweight gain and food consumption were not adversely affected at any dose level, and no significant clinical signs were observed. At necropsy on gestation day 20, no treatment-related effects were observed at any dose level. Parameters evaluated included post-implantation loss, live litter size, mean foetal bodyweights, foetal sex ratios and the mean numbers of corpora lutea and implantation sites. Differences from the control group values were slight and not statistically significant.

External malformations were observed in 4 foetuses in the 500 mg/kg bw/d group, anophthalmia (bilateral) was observed in 3 foetuses, but was not statistically significant. One of the 3 foetuses, in addition, had a facial cleft and exencephaly (without open eyelid), and one remaining foetus had exencephaly alone without open eyelid. Although the values were not statistically significant, the incidence of exencephaly exceeded the control values. One foetus was hydrocephalic with a dome-shaped head. Since there were no malformations observed in the 1000 mg/kg bw/d group foetuses, the malformations in the 500 mg/kg bw/d group were not considered to be HBCD-related. No other external malformations were noted. No external developmental variations were observed in any of the foetuses at any dose level.

There were no soft tissue malformations in any of the examined foetuses at any dose level. Soft tissue developmental variations were observed in the control, 250, 500 and 1000 mg/kg bw/d groups respectively. One foetus in each of the 500 and 1000 mg/kg bw/d groups had a retro-oesophageal right subclavian artery. These single occurrences were not considered to be related to HBCD administration.

A skeletal malformation (vertebral centra anomaly that consisted of fused lumbar centra) was noted in one foetus in the 500 mg/kg bw/d group. No other skeletal malformations were observed. Skeletal developmental variations consisting of unossified sternebrae (2 foetuses), unossified cervical centrum (1 foetus) and rudimentary ribs (2 foetuses) occurred in both control and all dose groups. No relationship to treatment was evident.

There was no statistically significant increase in the incidence of visceral or skeletal malformation in any of the treated groups compared to the control group. The incidence of foetal developmental variations in the treated groups was similar to that observed in the control group and was not considered to be related to treatment with HBCD. Based on the above findings, HBCD was not considered to be a developmental toxin. In this study the NOAEL for maternal and developmental toxicity was 1000 mg/kg bw/d.

Saegusa et al. (2009) investigated the developmental toxicity of HBCD in rat offspring after maternal exposure from mid-gestation through lactation.

In the study, pregnant Crj:CD rats given 0, 100, 1000 or 10 000 ppm of HBCD from GD 10 until the day 20 after delivery showed increased relative thyroid weights and an increased incidence of thyroid follicular cell hypertrophy at 10 000 ppm as compared to controls. At 100 and 1000 ppm the incidence of thyroid follicular cell hypertrophy showed a tendency to increase, although this change was not significant.

No offspring parameters revealed any abnormality; however, an increased relative weight of the liver was observed at 10 000 ppm in both sexes. In male offspring, serum T3 levels were decreased at this dose while serum T4 concentration was unaltered. No histopathological thyroidal changes were found in these animals. A statistically significant increased incident of diffuse vacuolar degeneration of liver cells was observed at 10 000 ppm in both sexes at PND 20. At adult stage, slight reduction in serum T3 concentration as well as slight increase in relative thyroid weight were found from a dose of 1000 ppm, suggesting a slight but sustained hypothyroidism until adult stage beginning at a dose of 1000 ppm.

Among the brain morphometric parameters, the oligodendroglial cell number were found decreased in the offsprings at 10 000 ppm, while no changes were observed in neuronal migration parameters. These results suggest a mild effect of HBCD on ologodendroglial development at 803 to 2231 mg/kg bw/d of maternal exposure, probably through a hypothyroidism-related mechanism.

In the 1-generation study conducted by Van der Ven et al. (described above), no differences in sex ratios were observed in F1 litters. F1 bodyweights, taken at PND 4 and then weekly, showed a dose-dependent decrease, varying between 7% and 36%.

The time to vaginal opening was delayed in the female pups at the top dose, but there was no effect on perputial separation in male pups or on AGD of female F1 pups. The weight of testes was decreased with a low BMDL of 11.5 mg/kg bw/d. Though detailed data were not provided, the authors state that further analysis of testes weight also showed a significant dose response. Effects with high BMDLs were decreased kidney and thymus weights in both sexes and decreased weights of adrenals, heart, brain and prostate in F1 males.

Bone scans to measure trabecular bone parameters revealed a decrease in trabecular bone mineral density in F1 females. Other affected bone parameters were decreased length in males and females and decreased total mineral content. A clear dose response was not apparent.

There were also marked decreases in liver apolar retinoid levels in male and female rats at all doses, with BMDLs of 24.3 and 21.7 mg/kg bw/d for total amount of apolar retinoids per liver, for males and females, respectively. A dose response was evident only at the higher levels of exposure.

Decrease in testes and prostate weight in male weanlings and delayed vaginal opening in females were considered to be treatment-related and not related to decreased bodyweights, since these effects were observed at a lower BMDL than the BMDL for the weight decrease. These observations also indicate that HBCD could have a hormone disrupting effect as the reporductive organ weights and time to vaginal opening are sex-hormone-related.

The most sensitive effects in this study were decreased mineral density in trabecular bone in F1 females, decreased concentration of apolar retinoids in liver in F1 females and altered immune response in F1 males, all of which were induced at low doses. Marked decreases in liver apolar retinoids were observed in both male and female rats.

The 2-generation study in rats conducted by Ema et al. (described above) also looked at developmental effects of HBCD. In F0 females at 1500 ppm, there was a significantly longer gestation length and lower sex ratio compared to controls. In F1 and F2 pups, there was no significant difference in the anogenital distance (AGD) or AGD per cube root of bodyweight ratio or any difference in age at preputial separation in males or vaginal opening in females when compared to controls. Significant decrease in bodyweight was observed in F1 male weanlings and F2 male and female weanlings at 15 000 ppm compared to controls, but the decrease was not seen in adult F1 animals.

The viability of F2 generation pups was not affected at day 0, but it was significantly reduced on day 4 and day 21 of lactation in the 15 000 ppm group. A similar effect, though not statistically significant, was also observed in the 1500 ppm group. The reduction of viability in the 15 000 ppm group was mainly due to a total loss of 8 litters by the F1 dams up until day 18 of lactation without any clear clinical signs of toxicity in the dams. A high and dose-dependent pup mortality during lactation was thus observed in the F2 generation (35% at 15 000 ppm HBCD and 15% in the 1500 ppm group). In the F2 generation, the incidence of pups showing eye opening was lowered at 1500 and 15 000 ppm, compared to controls.

Based on low bodyweights of pups and high mortality during lactation at the mid and high doses in the 2-generation reproductive study, a NOAEL of 150 ppm (10.2 mg/kg bw/d) was established.

Overall summary of reproductive effects of HBCD

The 1- and 2-generation reproductive toxicity studies in rats did not show any marked effects of HBCD on the fertility of animals. In the 2-generation study, the fertility index was lower in the treated rats but not significantly different from those of control rats. There were no changes in copulation index, gestation index or the number of implantations. Although primordial follicles were significantly lower in mid- and high-dose F1 females in this study, they were still within the range of historical control values from 4 earlier studies performed in the same laboratory that performed the current study and with the same species of rats. There were also large variations in the data and a lack of clear dose-response relationship. In a 28 d repeated dose toxicity study, a low number of follicles and ripening follicles in the ovaries was reported at the extremely high level of oral exposure of 4700 mg/kg bw/d. These effects were not observed at other high-dose levels of 940 and 2400 mg/kg bw/d.

A clear NOAEL for effects on fertility could not be established in any of the studies. The reduction in fertility index in male and female rats, although not statistically significant, and lower primordial follicles in F1 females at 1500 ppm and higher HBCD levels in the Ema et al (2008) study suggest a NOAEL of 150 ppm or 10.2 mg/kg bw/d.

Three developmental studies and a 1-generation and 2-generation reproductive studies assessed the effects of HBCD on development in animals. In the 3 developmental studies pregnant rats were administered HBCD during the gestation period, with doses of up to 1000 mg/kg bw/d. No significant developmental effects were noted in the animals in these studies. There was no adverse effect on the number of corpora lutea, implants, resorptions, live fetuses, sex ratio, or body or placental weights. Although one of the studies (Stump, 1999) revealed external malformations at 1 dose, these were not considered to be treatment-related, as no malformations were observed at the highest dose. No soft tissue malformations were noted in any of the examined foetuses at any dose level.

In the 1-generation reproductive study, bodyweights of F1 pups showed a dose-dependent decrease. The time to vaginal opening in females was delayed at the top dose and male pups showed increased AGD at postnatal day (PND) 4. Testes weights were decreased at low doses with a significant dose-response relationship. Systemic effects at high doses of HBCD were decreased kidney and thymus weights in both sexes and decreased adrenals, heart, brain and prostate weights in F1 males. A decrease in bone mineral density in females and decreased total mineral content, total area and cortical thickness in males in the femur and tibial bones were noted. Both male and female pups showed marked dose-dependent decreases in liver apolar retinoid levels.

Retinoids regulate the transcription of numerous target genes and play a vital role in a multitude of processes such as developmental programming, skeletal morphogenesis, embryonic growth, sex differentiation, vascularization and reproduction. It is therefore postulated that the developmental effects found in the present study may be linked through retinoid homeostasis, although further mechanistic investigation is necessary to substantiate such a hypothesis.

In the 2-generation reproductive study, a high and dose-dependent pup mortality during lactation was observed in the F2 generation (35% in the high-dose group and 15% in the mid-dose group) in the absence of clear clinical signs of toxicity in the dams. Decrease in bodyweights of male F1 and F2 pups and female F2 pups were also noted at the high dose. In F2 generation, the incidence of pups showing complete eye opening was lowered compared to controls. A few F2 pups in the HBCD-treated groups also failed to complete the reflex response test. Other effects noted in F2 weanlings were decreased weights of kidney, brain, spleen, adrenal, epididymis, ventral prostate and ovary at mid and high doses.

Effects on pup mortality, body and organ weights and liver apolar retinoids in the reproductive studies indicate that HBCD has adverse effects on development and pup viability in rats.

Based on low bodyweights of pups and high mortality during lactation at the mid and high doses in the 2-generation reproductive study, a NOAEL of 150 ppm (10.2 mg/kg bw/d) was established.

8.2.8 Other effects

Developmental neurotoxicity

A neurotoxicity study was conducted in mice to determine the effects of exposure to HBCD during brain development (Eriksson et al., 2006). Neonatal male NMRI mice (8–10 animals/group) were exposed on day 10 to HBCD [0.9 or 13.5 mg (1.4 or 21μmol)/kg bodyweight] as a single oral dose by gavage. The day of administration (PND 10) corresponds to the peak in rodent brain growth spurt (BGS), when the brain undergoes several fundamental changes such as dendritic and axonal out-growth and the establishment of neural connections (Davison & Dobbing, 1968). At the age of 3 months, neurobehavioural development of the mice was assessed by observing their spontaneous behaviour, learning and memory capability.

Spontaneous behaviour was studied in a total of 10 animals randomly selected from 3 different litters from each treatment group. Motor activity was measured for 3 periods of 20-minute durations in an automated device consisting of cages placed within 2 series of infrared beams. Locomotion (horizontal movement), rearing (vertical movement) and total activity (all types of vibrations within the test cage) were monitored. Learning and memory, which are hippocampus-dependent spatial learning tasks, were observed in a Morris water maze. Male mice from 3 different litters (5 trials/animal/d) were observed trying to find a submerged platform for 4 consecutive days. On the fifth day, the platform was moved and the relearning ability of finding the platform was registered.

Spontaneous behaviour of mice exposed to HBCD in both dose groups was significantly altered, manifested as reduced habituation with initial hypoactivity followed by hyperactivity as compared to controls. In the high-dose group (13.5 mg/kg bw/d) all 3 parameters (locomotion, rearing and total activity) were significantly affected during the first and last 20-minute period. Mice in this group were less active as compared to controls. In the low-dose group (0.9 mg/kg), locomotion and rearing was significantly decreased by a factor of approximately 1.3 during the first 20-minute period, but there were no effects in later measurements or in total activity.

Water maze tests (Morris water maze type) assessed the ability of adult mice to learn and memorise spatial navigation tasks. During the acquisition period (1–4 d) of spatial learning ability, treated and untreated mice significantly improved their ability to locate the platform. On the fourth day, the mean latencies of mice exposed to 13.5 mg/kg bw/d were significantly longer than in the control animals and in mice given 0.9 mg/kg bw/d. The 4 d acquisition period was followed by reversal learning on the fifth day. There was a significant difference in search time between the control and the treated mice and the mice given 13.5 mg/kg bw/d HBCD took longer to find the new position of the platform.

The effects observed in this study were increased in a dose-related fashion although only 2 exposure groups were used, and the behavioural alterations were induced at doses that did not affect the weight gain or evoke any clinical signs of ill-health.

Neonatal exposure to HBCD affected spontaneous motor behaviour, learning and memory processes in adult mice. Results indicate that HBCD can cause developmental neurotoxic effects at low exposure levels, with clear effects on all parameters at 13.5 mg/kg bw/d, and on some effects at 0.9 mg/kg bw/d. Given that only 2 doses were used, NOAEL could not be established. A LOAEL of 0.9 mg/kg bw/d was derived from this study.

In the 2-generation study in rats (Ema et al., 2008) described in Section 8.2.7.1, some effects on the completion of eye opening and the surface righting reflex response were observed in F1 and F2 pups; however, this was not consistent over generations or sexes and hence was not considered substance-related. There were no significant differences in spontaneous locomotor activity between the control and HBCD-treated groups.

The effect of HBCD on the binding of neurotransmitters to plasma membrane receptors was studied by Mariussen and Fonnum (2003). A synaptosomal preparation of rat brain (Wistar strain) suspended in sucrose was incubated at 25 ºC for 15 minutes in absence or presence of 2-20 µM HBCD. Radiolabelled dopamine, glutamate or γ-amino-n-butyric acid (GABA) were added to the suspension to start the binding and the reaction was terminated by adding cold BSA (bovine serum albumin) solution and rapidly filtering the whole suspension. The filters were then dissolved and counted for retained radioactivity in a liquid scintillation spectrophotometer.

The results indicated that HBCD inhibited uptake of neurotransmitters by synaptosomes and uptake of dopamine by synaptic vesicles. Dopamine and glutamate uptake was inhibited at low concentrations of HBCD. Kinetic analysis with dopamine binding indicated that HBCD inhibits dopamine uptake non-competitively.

These results suggest that HBCD might have a neurotoxicological potential; however, for the neurodevelopmental studies described above, the study methods used were not a standard protocol and the tests did not use a standard test strain, leading to difficulty in interpretation of results.

Endocrine disrupting effects

There is increasing evidence that some BFRs may have endocrine disrupting (ED) effects. Studies examining ED activity of HBCD via thyroid hormone levels and liver enzymes have been conducted. In addition, a few neurodevelopmental studies and related neurological mechanistic studies have been conducted.

Effects on thyroid hormones

Repeat dose studies showed marked effects of HBCD on the thyroid hormone (TH) axis, including increased weight of thyroid glands and the pituitary. Other effects were decreased total thyroxine (TT4), increased immunostaining for TSH and induction of T4-UDPGT in the liver. Most of these effects were observed in female rats and were consistent with higher HBCD liver concentrations in females versus males.

In the 2 28 d studies discussed in Section 8.2.4.1, HBCD induced increased thyroid gland weight and decreased T4 in rats (females only). Other effects on TH concentrations noted were the feedback on the TH axis, which showed increased pituitary weight and increased immunostaining of TSH in the pituitary and thyroid follicle cell activation in females only. The gender differences may be related to gender-specific differences in uptake and/or metabolism of HBCD as noted by the higher internal concentrations of HBCD in the liver in females. At necropsy, microfollicular hyperplasia and increased activity of the epithelium in the thyroids were revealed.

Similar effects were noted in the 90 d oral study, where HBCD induced a slight increase in the incidence of minimal follicular cell hypertrophy in the high-dose males (1000 mg/kg bw/d) and minimal or mild hypertrophy in the high-dose females. Serum concentrations of T4 and TSH were dose-dependently affected in all dose groups of both sexes, with decreases in T4 (21% and 37% in the high-dose females and males, respectively) and increase in TSH (approximately tenfold).

Two mechanisms are known to be responsible for the decrease in T4: induction of UDPGT, which conjugates with T4 and increases its removal; and competitive interaction of hydroxylated metabolites with TTR, the major T4 binding protein in rodents, resulting in release of T4 and removal by normal metabolic processes. The latter process may be of limited importance, as no hydroxylated metabolites of HBCD were detected following dosing with HBCD.

Postulated mode of action of HBCD on thyroid system

The mechanisms by which HBCD induces effects on the thyroid system in rats is not fully understood. It is suggested that HBCD evokes a detoxification response in the liver via enzyme induction that leads to increased excretion of T4. Decrease in T4 levels leads to increased production of T3 and increase in thyroid gland weight.

Key events

The key precursor events associated with effects on thyroid system are assumed to be induction of hepatic enzymes involved in the metabolism of T4 and T3. Indeed, the T4-UDP glucoronyl transferase has shown to be induced in rats (both sexes) treated with HBCD. This is also supported by increased liver weight in these animals, associated with histopathological changes such as hepatocellular basophilia, suggestive of induction of endoplasmic reticulum.

Dose-response relationship

Available data show a non-linear dose-response pattern for the key events (at least in the female rats). In the 28 d study (Van der Ven et al., 2006), the BMDL for induction of hepatic T4-UDP glucoronyl transferase was 4.1 mg/kg bw/d, followed by the BMDL for liver weight increase of 22.9 mg/kg bw/d. The BMDL for pituitary weight was 29.9 mg/kg bw/d. Increased thyroid weights were also noted at 30 mg/kg bw/d and enhanced immunostaining of TSH in the pituitary and thyroid follicle cell activation were also seen above these doses in female rats. These observations support the hypothesis that hepatic enzyme induction and increased metabolism of T4 are the initial stages of the effects on the thyroid system.

The relevance of thyroid hormone depletion to humans, particularly as it relates to the TTR competition mechanism, is not clear, as there are major differences in T4 transport between rodents and humans, with humans having a different major T4 carrier. In humans, the major carrier is thyroxine binding globulin (TBG), which, in addition to differing in terms of competition mechanisms, gives tighter binding than TTR (McDonald, 2002), potentially reducing the availability of T4 for gluconuridation. Both TTR competition differences and differences in the availability of T4 for conjugation and elimination lead to a difference in the toxicokinetics of thyroid hormone mediated effects between humans and rodents. This is important, as thyroid hormone depletion may have a major role in a number of the toxicological effects, particularly the neuro-developmental effects, seen in animal models.

Liver enzyme induction is likely the most important reason for liver weight increase and probably also for effects on thyroid system. Enzyme induction is relevant to humans too. It is possible that HBCD could also affect the thyroid system via other mechanisms, such as by binding to thyroxin-binding sites. Evidence of HBCD interference with the binding of thyroxine and other hormones to their respective receptors has been shown in studies looking at endocrine disrupting effects of HBCD. However, this needs further investigation.

Because of the reported effects of HBCD on the thyroid system and the importance of thyroid hormones in the development of the auditory system (Ng et al., 2004), Lilienthal et al. (2009) studied the effects of HBCD exposure on the auditory function. Using the haloperidol-induced catalepsy and brainstem auditory evoked potentials (BAEPs) methods, Lilliental et al. assessed the dopamine-dependent behavior and hearing function in adult male and female offspring. Rats were exposed to 0, 0.1, 0.3, 1, 3, 10, 30 or 100 mg HBCD/kg bodyweight via the diet before mating, during mating, gestation, lactation (adult rats), and after weaning (offspring). Reduced latencies to movement onset were observed mainly in female offspring, indicating influences on dopamine-dependent behavior. The overall pattern of BAEP alterations, with increased thresholds and prolonged latencies of early waves, suggested a predominant cochlear effect and therefore potential to affect auditory system in exposed animals. Effects were dose-dependent with lower bounds of benchmark doses (BMDL) between <1 and 10 mg/kg bodyweight for both catalepsy and BAEP thresholds. However, the tissue concentrations of HBCD at the BMDL values obtained in this study were 3 to 4 orders of magnitude higher than current exposure levels in humans.

Van der Ven et al. (2009), however, did not observe any effects of HBCD on any of a series of thyroid parameters studied in F1 generation rats in a 1-generation reproductive study (see Section 8.2.7.1). They have attributed this to adaptive mechanisms in the F1 animals after their long-term exposure to HBCD or to the disruption of TH system early in life.

HBCD has been shown to affect circulating thyroid hormones, and studies indicate that an insufficient supply of thyroid hormones in the early postnatal period may affect the function of the apical part of the cochlea and thus hearing in the lower frequency range. The BAEPs test was therefore selected to study this effect further. Lilienthal et al. (2007) studied the possible effects of HBCD on auditory function in rats. They specifically monitored the BAEPs following prenatal and postnatal exposure to HBCD. Nine dose levels of HBCD, ranging from 0 to 100 mg/kg, were used. Exposure design was chosen to allow benchmark analysis of the results. Exposure started in parental rats and was continued throughout gestation and lactation and after weaning in offspring. BAEPs were recorded in adult offspring. BAEPs were affected by HBCD only in male offspring. Thresholds were elevated and latencies prolonged in the low frequency range, but latency prolongation was not more pronounced on later waves. The authors postulated that this suggests a predominant cochlear effect. In addition, haloperidol-induced catalepsy was examined in HBCD exposed rats, because of known interactions of thyroid hormones with the dopaminergic system. Results revealed dose-related decreases in latencies to movement onset. However, the endocrine relation remained unclear in HBCD-treated rats, as serum hormone levels were not changed by exposure. Benchmark analyses were used to determine critical effect doses (CED) and their BMDLs. BMDL values were in the range from 1 to 10 mg/kg and from <1 to 25 mg/kg for auditory effects of TBBPA and HBCD, respectively. BMDL values for catalepsy were in the range from 2 to 16 mg/kg in HBCD-exposed rats.

In-vitro studies of endocrine-disrupting effects

Hamers et al. (2006) performed a systematic in-vitro screening of the potential ED potencies of 27 BFRs, including HBCD, under the Flame Retardants Integrated Risk Assessment for Endocrine Effects (FIRE) program. For all test compounds, the potency to interact as an agonist or antagonist with the arylhydrocarbon (Ahr), the estrogen receptor (ER), the androgen receptor (AR) and the progesterone receptor (PR) was determined in the AhR-, ER-, AR- and PR-CALUX bioassays (chemically activated luciferase gene expression). These assays make use of reporter cell lines carrying a luciferase gene under the transcriptional control of response elements for activated receptors. The 4 CALUX bioassays were performed according to protocols supplied by the bioassay manufacturing company. In addition, 2 measures of thyroid-disrupting potency (competition with thyroid hormone precursor, thyroxine (T4), for T4-binding sites on the carrier protein, transthyretin (TTR) and mimicking of T3) were also investigated.

The in-vitro screening demonstrated that ED potencies were shared by a wider set of BFRs. HBCD also displayed PR-antagonistic potency. Based on their receptor binding effects, BFRs were classified into 5 different ED toxicity profiles (Clusters). HBCD was placed in Cluster III, which consisted of BFRs with moderate to high T3-potentiating activity and low to moderate anti-androgenic and anti-progestagenic activity in combination with low to moderate AhR-antagonistic activity. In conclusion, in-vitro bioassays proved to be useful tools in screening the possible ED potency of BFRs, and grouping them into clusters with similar toxicity profiles, and to help in interpreting in-vivo results. The in-vitro assays further demonstrated that different BFRs have different metabolisation rates. The assays further indicated that metabolism and photochemical transformations by UV light may be important prerequisites for BFRs to exert their ED mode of action.

To determine the ED potency of HBCD, Yamada-Okabe et al. studied the effect of HBCD on the thyroid hormone receptor-mediated (TR-mediated) gene expression in HeLaTR cells that stably expressed the human TRα1 (Yamada-Okabe et al., 2005).

To monitor the TR-mediated gene expression, human liver 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 was driven by TR. The transfected HeLaTR cells were treated for 2 d with HBCD (3.12, 6.25, 12.5 and 25 μM), in the presence or absence of T3 (50 ng/ml). The cells were thereafter lysed and analysed for luciferase activity. It was found that 4,4'-diiodobiphenyl (DIB), markedly, and HBCD, 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. All the tested concentrations of HBCD increased TR-mediated luciferase activity significantly (1.6–1.8-fold) in the presence of T3. The effects were not due to toxicity or proliferation, as HBCD was tested in the MTS-cell growth assay prior to analysis, and concentrations that were cytotoxic were not further tested.

The TR is 50% homologous with the estrogen recetor (ERα). HBCD was therefore also tested for ER-mediated gene activation in MCF7 cells. HBCD did not affect the E_2-induced expression of the luciferase gene that was linked to the estrogen responsive element in MCF7 cells indicating that HBCD affects only TR-mediated gene expression.

Effects on enzyme induction

The effects of HBCD on enzyme induction in rats were studied by Germer at al. (2006). Hepatic mRNA and microsomes isolated from rats in the 28 d oral study (Van der Ven et al., 2006) were used to monitor hepatic cytochrome P-450 (CYP 450) levels and CYP 450 activity.

The main focus of the study was to evaluate the effect of HBCD on the CYP 1A, CYP 2B and CYP 3A4 enzyme families (enzymatic activity, mRNA and protein level). The enzyme activity was assessed with the following methods: 7-ethoxyresorufin *O*-deethylase assay (EROD), 7-pentoxyresorufin-*O*-dependentylase (PROD), and luceferin benzylether debenzylase (LBD)-assay, believed to represent CYP 1A, 2B, and 3A4 enzymatic activity, mRNA levels (CYP 1A2, 2B1, 3A1/3). The amount of enzyme protein (CYP 1A1/2, 2B1/2, 3A1) was measured according to standard RT-PCR and Western blotting procedures.

HBCD treatment led to a significant induction of CYP 2B1 mRNA, CYP 2B1/2B2 protein and PROD activity, suggesting a phenobarbital-type of induction. The induced CYP 2B enzymatic activity in males was confirmed on mRNA and protein level (fivefold and threefold induction at the highest dose, respectively). Furthermore, a significant increase in CYP 3A1/3A3 mRNA, CYP 3A1 protein, and luciferin benzylether debenzylase (LBD) activity was found, being more pronounced in females than in males. The effect on CYP 3A1/3A3 mRNA was significant in female rats at a daily dose of 3.0 mg/kg bodyweight and above. HBCD exhibited no effects on CYP 1A2 mRNA, CYP 1A1/1A2 protein, or microsomal 7-ethoxyresorufin O-deethylase (EROD) activity suggesting lack of activation of the aryl hydrocarbon receptor. These findings suggest that HBCD did not cause a general enzyme induction in rats. The detected HBCD-induced effects were rather specific, with induced CYP 2B and CYP 3A4 in males and females respectively.

The authors suggest that induction of these drug-metabolising enzymes in rats is 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.

In the 1-generation reproduction study (Van der Ven et al., 2009), effects of HBCD on the production of sex steroid hormones were assessed by analysing the activity of CYP 19 (aromatase), the key enzyme for oestrogen synthesis and by measuring dehydroepiandrosterone (DHEA), a product of CYP 17 and a key enzyme in androgen synthesis. Effects on steroidogenesis included induction of CYP 19 in the ovary and CYP 17 in the adrenals in the mid-dose groups in the 28 d study. However, the results were not statistically significant. Other effects noted were the induction of T4-UDPGT.

In conclusion, the effects on liver enzymes induction seen in both in-vitro and in-vivo studies indicate that HBCD has induction potential indicative of a phenobarbital type inducer, characterised by increased PROD and UDPGT activities (McClain et al., 1989).

Immunotoxicity

The 1-generation reproductive toxicity study described earlier (Ven der Ven et al., 2009) also tested the effect of HBCD on the immune system. Immunotoxicity of HBCD was assayed by testing the immunisation efficacy of sheep red blood cells (SRBC) in a separate cohort of F1 animals, starting at the age of 8 weeks. 4 male rats per dose group (0, 0.1, 0.3, 1,3, 10, 30 or 100 mg/kg bw/d HBCD), from different litters, were injected

intraperitoneally with 2×10^9 SRBC on day 0 and day 15 (booster). Blood was drawn on days 0 (control), 7 (for IgM analysis) and 21 (for IgG analysis) by orbital punction or terminal bleeding. Antibody titers in the serum were determined with ELISA.

Five animals from each dose group were necropsied and a standardised part of the spleen was taken for fresh analysis of (immune) cell subpopulations and/or natural killer (NK) cell activity. Popliteal lymph nodes were dissected with high precision and measured with an analytical balance.

The immunisation assay against sheep red blood cells revealed no change in the initial immunisation response when read specifically for IgM after 7 d. There was, however, a significantly increased specific IgG response after 21 d. The NK activity test showed no effect. On the other hand, spleen cell analysis revealed an increase of the NK cell fraction at higher doses.

In the peripheral blood of male littermates of the animals used for immunisation, there was an increased fraction of neutrophilic granulocytes. There were also significant dose responses for decreased lymphocyte fraction, a decreased whole white blood cell count in the blood and an increased white blood cell count in the bone marrow, although not statistically significant. The same is true for decreased thymus weight in both sexes, and for increased weight of popliteal lymph nodes in males. There were no discernible exposure-related histopathologic changes in the thymus and popliteal lymph nodes. In the spleen, on the other hand, the marginal zone showed enlargement, with a significantly higher frequency in top-dose animals compared with control animals.

The above parameters are usually intended for screening for potential immunotoxicity and, based on the changes observed in these parameters, the authors of the study concluded that HBCD exerts effects on the immune system.

In order to study the mechanism by which HBCD decreases the lytic function of human natural killer (NK) cells (as observed in the above study), Hinkson and Whalen (2009) exposed NK cells to various concentrations of HBCD for 24 and 48 h and 6 d before determining lytic function and ATP levels. ATP levels and lytic function were also determined in NK cells that were exposed to HBCD for 1 h followed by 24 and 48 h, and 6 d in HBCD-free media. The results indicated that exposure of NK cells to 10 µM HBCD for 24 h causes a very significant decrease in both NK cell lytic function and ATP levels (93.5% and 90.5%, respectively). Exposure of NK cells to 10 µM HBCD for 1 h followed by 24 h in HBCD-free media showed a progressive and persistent loss of lytic function (89.3%) as well as a decrease in ATP levels (46.1%). The authors concluded that HBCD exposures decreased lytic function as well as ATP levels. Importantly, a brief (1 h) exposure to HBCD can cause a progressive loss of lytic function over a 6 d period.

To further investigate the possible causes of the HBCD-induced decreases in lytic function, Hinkson and Whalen (2010) examined the effects of HBCD on the ability of NK cells to bind to their targets and express cell surface markers.

NK cell binding to their targets is the first step of achieving target lysis. Thus, if HBCD were to interfere with binding that could at least in part explain the HBCD-induced loss of lytic function. Also, since NK cells use specific cell-surface proteins to recognise and bind to their targets, it was argued that any observed decrease in binding could be explained by an accompanying decrease in specific cell-surface protein expression.

NK cells isolated from human blood were exposed to different concentration of HBCD. NK cell binding was tested by the conjugation assay using NK-susceptible K562 cells. Exposures of NK cells to 2.5, 5 and 10 μM HBCD for 1 h followed by a 24-h incubation in HBCD-free media caused significant decreases in their binding function (43%, 65% and 79%, respectively). When NK cells were exposed to 2.5, 5 and 10 μM HBCD for 1 h followed by a 48 h period in HBCD-free media, there were also significant decreases in their binding function (48%, 48% and 82%, respectively).

The loss of binding function of NK cells was compared with the loss of their lytic function seen in a previous study (Hinkson & Whalen, 2009). The loss of lytic function after 24 h exposures to HBCD was somewhat greater at some concentrations of HBCD than the loss of binding function. A 48 h exposure to 2.5 μM HBCD caused NK cells to lose 75.6% of lytic function and 37.3% of binding function. A 6 d exposure to HBCD resulted in a large decrease in both lytic and tumor binding function.

To investigate if the loss of binding function was due to loss of specific cell surface proteins utilised by NK cells to recognise and bind to their targets, the authors examined the cells' capacity to alter expression of several cell surface proteins. There was a very significant loss of the cell surface proteins CD16 and CD56 in NK cells exposed to 10 μM HBCD for 24 h (CD16 and CD56 have been shown to be important in binding of NK cells to target cells for their lytic function: Mandelboim et al., 1999).

The levels of HBCD found in human blood and serum (0.3 nM in serum) are lower than those where the effects on NK lytic function was seen (1 μM); however, the authors argue that there is a possibility of much higher accumulations in individuals who work in the application of HBCD to the various products in which it is used.

These studies indicate that HBCD decreases the lytic function of human NK cells that persist even after its removal from the cell medium. Since NK cells are a primary immune defence against tumor cells and virally infected cells, interference with the normal function of these cells can have serious consequences. Results from these studies need to be interpreted with caution as the protein binding assays and cell lysis experiments were carried out in an in vitro situation in NK cells isolated from human blood. There are always limitations to in vitro tests as the physiological milieu could also have effects on cell lysis and protein binding function of the NK cells. Additional in vivo studies are required to further investigate the effect of HBCD on immune system.

8.3 Effects on human health

No case reports on the potential effects of HBCD on humans are available. The only reported study on the effects of HBCD is a repeat insult patch test, discussed below.

8.3.1 Skin sensitisation

In a study to determine the sensitisation potential of HBCD in humans, one-inch squares of Tyvek T-12 1 treated with 10% HBCD were placed on the arms of 10 men or on the arms or legs of 10 women and held in place with tape for 6 d. After 2 weeks, new patches were applied for 48 h as a challenge test for skin sensitisation. The skin under the patches was examined after removal of the challenge patch, 2 and 6 d after the first application. No skin reactions were observed in any of the subjects tested (McDonnell, 1972).

8.4 Regulatory classifications based on hazard

This section discusses the classification of HBCD according to the Safe Work Australia *Approved Criteria for Classifying Hazardous Substances* (the Approved Criteria) (NOHSC, 2004) for health effects or, in the case of physicochemical hazards, the *Australian Code for the Transport of Dangerous Goods by Road and Rail* (ADG Code, 7th edition) (NTC, 2007). The Approved Criteria are cited in the NOHSC *National Model Regulations for the Control of Workplace Hazardous Substances* (NOHSC, 1994a) and provide the mandatory criteria for determining whether a workplace chemical is a hazardous substance.

Where adequate human data were unavailable, the classification for health hazards has been based on experimental studies (animal and in-vitro tests). In extrapolating results from experimental studies to humans, consideration was given to relevant issues such as quality of data, weight of evidence, metabolic and mode of action/mechanistic profiles, inter- and intra-species variability and relevance of exposure levels.

HBCD is currently not listed on Safe Work Australia's Hazardous Substances Information System (HSIS).[5]

8.4.1 Physicochemical hazards

HBCD is a white, odourless solid with a vapour pressure of $6.3\ 10^{-5}$ Pa @21 ºC. The boiling point could not be determined due to decomposition at high temperatures.

According to the ADG Code (NTC, 2007), HBCD does not meet the criteria for classification as a dangerous good on the basis of its physicochemical hazards. However, based on its environmental toxicity (Chapter 9), HBCD falls under Class 9, Packaging group III and UN number 3077 (environmentally hazardous substance).

8.4.2 Health hazards

Acute toxicity

No human data are available to determine the acute toxicity of HBCD. In 2 toxicity tests conducted in animals, one in rats and another in mice, no significant findings were noted at the end of the 14 d observation period in the animals administered the highest dose of 20 000 mg/kg bw/d. No mortalities were reported. The LD50 was determined to be >20 000 mg/kg.

No toxic effects and no mortalities were noted in an acute dermal study and a 14 d range finding study in rabbits administered 20 g/kg bw and 8 g/kg bw respectively as a single application under occlusion. The LD_{50} was determined to be >20 g/kg bw.

In acute inhalation studies, male and female rats exposed whole body to HBCD dust at concentrations of 200 to 202 mg/L from 1 to 4 h exhibited no mortalities, toxic effects or gross pathological changes at these doses. The LC_{50} was determined to be >200 mg/L.

[5] http://www.hsis.ascc.gov.au/Default.aspx

Classification

HBCD does not meet the Approved Criteria (NOHSC, 2004) for classification for acute oral, dermal or inhalation toxicity.

Irritation and corrosive effects

In 2 studies conducted on New Zealand rabbits, no significant skin irritant effects were noted in any of the animals treated with HBCD at the end of the observation period of 72 h and 48 h respectively. In a Human Repeat Insult Patch Test (HRIPT), no skin reactions were reported in humans exposed to 10% HBCD for 6 d.

In 3 eye irritation studies, no eye irritant effects were noted at the end of the observation period of 72 h in any of the rabbits following the administration of HBCD to the rabbits' eyes.

Classification

HBCD does not meet the Approved Criteria (NOHSC, 2004) for classification as a skin or eye irritant.

Sensitising effects

Conflicting results were obtained in studies using EU grade HBCD and Japanese marketed HBCD. The study results are provided in Table 8.5. In the maximisation test in guinea pigs using the EU grade HBCD, reaction at the challenge site was evaluated for up to 120 h. No reaction was noted in any of the test animals after patch removal at any of the observation periods. In contrast, in a maximisation test study using Japanese marketed HBCD, following the application of the chemical to 10 female albino guinea pigs, at various doses, positive results were obtained in 9 out of 10 animals using lower induction and challenge doses. In another study using Japanese marketed HBCD, 9 out of 10 animals were sensitised at the highest concentration of induction. In both studies with Japanese marketed HBCD, a clear dose-effect relationship was observed. Results of an LLNA conducted on mice treated with the EU grade HBCD at 2%, 20% or 50% concentrations were not consistent with dermal sensitisation, as the SI values were consistently around 1.0 at all doses tested. A value of >3.0 is considered positive for sensitization. The difference in the outcomes of the studies using the EU-marketed grade of HBCD and Japanese HBCD may be due to the fact that the Japanese marketed brands may contain impurities that have sensitising effects. As the Magnusson-Kligman study and the LLNA conducted with the EU marketed grade of HBCD were negative, it is reasonable to state that this grade of HBCD is not sensitising, and that any effects may be due to specific impurities in the Japanese grade.

Table 8.5. Summary of skin sensitisation studies

Test method	Test material	Result	Reference
Magnusson and Kligman	EU marketed HBCD	Not sensitising	Wenk, 1996
Magnusson and Kligman	Japan marketed HBCD	Sensitising to skin	Nakamura et al., 1994
Magnusson and Kligman	Japan marketed HBCD	Sensitising to skin	Momma et al., 1993

Local Lymph Node Assay (LLNA)	EU marketed HBCD	Not sensitising	Wolhiser & Anderson, 2003

In a human study in which 10 men and 10 women were treated with 10% HBCD, no skin reactions were noted in any of the subjects tested at the end of the observation period of almost 3 weeks.

Classification

HBCD does not meet the Approved Criteria (NOHSC, 2004) for skin sensitisation.

Effects from repeated or prolonged exposure

Five repeated dose dietary studies in rats and one in mice have been conducted with HBCD. Table 8.6 provides the results of these studies. Increase in absolute and relative liver and thyroid weights were consistently noted in these studies. In one 28 d oral study, induction of liver enzymes was also demonstrated leading to the suggestion that increase in liver weight is due to enzyme induction. The same study reported dose-related effects on the thyroid axis, i.e. decreased total thyroxin (TT4), increased pituitary and thyroid weight and thyroid follicle cell activation. The postulated mechanism for these changes is that the hepatic emzymes involved in the metabolism of T4/T3 are induced by HBCD, leading to increased excretion of T4 and consequently, by feedback mechanism, to induction of TSH and T3 production with thyroid weight increase.

In 2 90 d oral toxicity studies in Sprague Dawley rats, the clinical chemistry changes, although significant, were not considered of sufficient magnitude to be adverse, as they also occurred in otherwise clinically-normal animals and tended to be within or close to historical or control values. Minor histological changes in the thyroid and the increase in prostate weight were also noted.

Table 8.6. Summary of repeat-dose studies

Study	Dose	Effects	NOAEL
28 d dietary study in rats (Chengelis, 1997)	125, 350, 1000 mg/kg bw/d	Liver weight increased at all doses but remained high at 1000 mg/kg bw/d group after recovery period. No histological effects observed in the liver or thyroid in either sex.	350 mg/kg bw/d
28 d dietary study in rats (Zeller & Kirsch, 1969)	0, 940, 2410 and 4820 mg/kg bw/d.	Liver weight increase and thyroid hyperplasia at all doses in both sexes. No histological changes observed.	Not established
28 d dietary study in rats (Van der Ven et al., 2006)	0, 0.3, 1, 3, 10, 30, 100, and 200 mg/kg bw/d	Increased liver, induction of hepatic CYP 2B and CYP 3A4. Increased pituitary and thyroid weights. Decreased total thyroxin.	BMDL10 = 22.9 mg/kg bw/d; derived NOAEL: 10 mg/kg bw/d
90 d dietary study in rats (Zeller & Kirsch, 1970)	0, 0.16, 0.32, 0.64 or 1.28% in the diet (0, 120, 470 and 950 mg/kg bw/d)	Liver weight increases at all dose levels from the lowest dose (120 mg/kg bw/d) in both sexes. No histopathological effects were reported.	>950 mg/kg bw/d
90 d dietary study in rats (Chengelis, 2001)	0, 100, 300 and 1000 mg/kg bw/d	Liver weight increase at all doses. Thyroid weight increased in mid- and high-dose females. Serum T4 was decreased and TSH increased in all dose groups. Prostate weight increased in the 1000 mg/kg bw/d group.	100 mg/kg bw/d (LOAEL)
18-month oral study in mice (Kurokawa et al., 1984)	0, 13, 130 and 1300 mg/kg bw/d	Hepatocytic swelling, degeneration, necrosis, vacuole formation and fatty infiltration in liver, but these changes were not dose-related. No carcinogenic effects observed.	Not established

Classification

Increase in liver weight is generally considered an adverse effect, especially if it is accompanied by histological changes in the liver. However, in the case of HBCD, increases in the liver weights were mostly reversible and not accompanied by any histological changes. According to the Approved Criteria (NOHSC, 2004), substances with oral NOAEL of less than 50 mg/kg bw/d can be classified as "Harmful" with the risk phrase "R48 – Danger of serious damage to health by prolonged exposure". But the Approved Criteria also specifies that adaptive effects such as liver hypertrophy and enzyme induction do not justify classification with R48.

Based on the effects observed in repeat dose studies, it is concluded that HBCD does not meet the Approved Criteria (NOHSC, 2004) for danger of serious damage to health by prolonged exposure. The increase in liver weights and thyroid are considered adaptive responses related to the effects seen on liver enzymes and the thyroid hormone system, and are reversible following cessation of treatment. Thus, although liver effects are taken into account when characterising risk from HBCD, the effects are not significant enough to warrant classification of HBCD with R48.

Genotoxicity

In-vitro Ames studies conducted on HBCD showed no increases in the number of revertants when compared to the solvent control group, regardless of the presence of the S-9 mix. No statistically significant increases in chromosome aberrations were observed in the in-vitro mammalian cytogenetic test using human peripheral blood lymphocytes (HPBL). No chromosome damage (clastogenic) or impairment of chromosome distribution was noted in the mouse micronucleus test. In a study conducted to induce intragenic recombination in mammalian cells, statistically-significant dose dependence was noted in the Sp5 assay and SPD8 cells. However, since all the above tests were negative for mutagenicity, the mutagenic status of HBCD could not be determined on this assay alone.

Classification

HBCD does not meet the Approved Criteria (NOHSC, 2004) for classification as a genotoxin (R46, R68).

Carcinogenicity

In the only carcinogenicity bioassay reported, B6C3F1 mice exposed to HBCD (0, 13, 130 or 1300 mg/kg bw/d) via the diet for 18 months did not show any overt signs of toxicity. The exposed animals had hepatic changes (hepatocyte swelling, degeneration, necrosis, vacuole formation, fatty infiltration), but there was a poor correlation between these effects and the dosage. Incidences of liver carcinomas were within the normal range for this strain of mouse. This study was not conducted according to OECD guidelines. No carcinogenicity studies in rats were reported. The available data are insufficient to determine the carcinogenic potential of HBCD.

Classification

HBCD does not meet the Approved Criteria (NOHSC, 2004) for classification as a carcinogen (R45, R49, R40).

Reproductive effects

Effects on fertility

In a 2-generation reproductive toxicity study, no significant differences in copulation index, gestation index, pre-coital interval, number of implantation, delivery index or number of F1 pups delivered between the control and HBCD-treated groups were noted. HBCD did not affect sex-hormone-dependent events, such as weight of reproductive organs and estrous cyclicity. A small but dose-dependent decrease in fertility index was indicated only in F0 rats.

In F1 females, the number of primordial follicles in the ovary in F1 females was significantly decreased at 1500 and 15 000 ppm of HBCD; however, there was no clear dose response. Moreover, these reduced mean values were within historical control mean values of 4 earlier studies performed by the same laboratory and in same animal species. Although these findings suggest that HBCD is potentially toxic to reproduction, no adverse effects on other reproductive parameters in F1 dams, or on the numbers of implantations or F2 pups delivered, were noted. In addition, the effect on primordial follicles were seen at mid- and high-dose levels at which HBCD also exerted general systemic toxicity in animals, such as the liver and thyrois systems. Thus, while the data in the study of Ema et al. (2008) provide evidence of toxic effects of HBCD (in liver and thyroid at high doses), they do not provide sufficient evidence that HCBD affects fertility of rats. Based on these observations, HBCD cannot be classified as toxic to reproduction. The authors of the study suggest that a continuous breeding study of HBCD may be needed to clarify the reproductive toxicity of HBCD. In the 1-generation reproductive study, testis and prostate weights were found to be reduced in F1 generation rats, although no significant effects of HBCD treatment on reproduction endpoints (mating success, time to gestation and number of implantation sites) were noted in these rats. Effect of HBCD on prostate weights was also noted in a 90 d oral study in rats, indicating a common effect, probably through the endocrine system.

In a 28 d repeat-dose study, female rats exposed to HBCD had inhibited oogenesis and follicles but at very high doses (4700 mg/kg bw/d). No effects were noted in male rats exposed to the same highest dose, except for small inner sexual organs.

Classification

Based on the lack of effect of HBCD on the main reprotuctive parameters (copulation index, fertility index, number of implantations and speramtogenesis) and sex-hormone-dependent events and lack of dose response in some of the observed changes (changes in the number of primordial follicles), HBCD does not meet the Approved Criteria for classification into category of substances that affect fertility (R62).

Effects on development

In the 1-generation study in rats, treatment-related decrease in the weights of the testis and prostate in male weanlings and delayed vaginal opening in female weanlings were noted, along with marked dose-dependent decreases of apolar retinoid levels in liver of F1 animals. Decrease in trabecular bone mineral density was also observed in F1 females.

In another one-generation developmental study exposure of female rats, from mid-gestation through lactation, led to HBCD-induced degeneration of liver of female and male offspring. A decrease in serum T3 and increase in TSH and a reduction in brain oligodendrocytes in male offspring were noted.

In the 2-generation study (Ema et al., 2008), bodyweight of F1 and F2 male weanlings were significantly decreased in the HBCD-treated group as compared to controls. Increased mortality of F2 generation from day 4 till day 21 postpartum was observed in the highest-dose group. Increase of relative weight of testis was also observed in F1 weanlings at all exposure levels however no effect was seen on weight of testis of F1 adults.

Delayed development in F2 pups was indicated by the reduced incidence of male and female pups showing eye opening on PND 14. The development of basic reflexes was also affected at the highest dose, leading to shorter time response in the surface righting reflex in F1 male pups on PND 5 at higher dose levels.

There are also indications of developmental neurotoxic effects in two different studies with limitations. In the study by Lilienthal et al. (2009), effects of HBCD on hearing function and dopamine dependent behaviour were observed in the adult offsprings of HBCD-treated rats. In the study by Eriksson et al. (2006), effects on spontaneous behaviour, manifested as reduced habituation with initial hypoactivity, followed by hyperactivity in a novel environment was observed in 3-month-old mice that had received a single dose of HBCD on PND 10.

Reproductive and developmental toxicity studies are summarised in Table 8.7.

Table 8.7. Summary of fertility and developmental toxicity studies

Study	Dose	Effects	NOAEL
<u>Fertility effects</u>			
One-generation reproductive study in rats (Van der Ven et al., 2009)	0, 0.1, 0.3, 1.0, 3.0, 10.0, 30 or 100 mg/kg bw/d	No dose-response effects on mating success, time to gestation, number of implantation sites and litter size.	Not established
Two-generation reproductive study in rats (Ema et al., 2008)	0, 150, 1500 or 15 000 ppm	Decrease in fertility index in F0 rats (not statistically significant); decrease in the number of primordial follicles in F0 and F1 rats at mid and high dose (not dose-dependent).	150 ppm (10.2 mg/kg bw/d)
<u>Developmental effects</u>			
Developmental toxicity study in rats (Murai et al., 1985)	0, 7.5, 75 or 750 mg/kg bw/d	No developmental effects were noted. Increased liver weight observed in dams at 750 mg/kg bw/d and above.	750 mg/kg bw/d for fetal toxicity 75 mg/kg bw/d for maternal toxicity
Developmental	0, 250, 500	Skeletal variations (unossified	Not established

toxicity study in rats (Stump, 1999)	or 1000 mg/kg bw/d	sternebrae, unossified cervical centrum and rudimentary ribs) occurred in all dose groups as well as in controls.	
Developmental toxicity study in rats (Saegusa et al., 2009)	0, 100, 1000 or 10 000 ppm	Hypothyroidism until adult stage. No abnormality in any offspring parameters.	100 ppm, for maternal toxicity (8.1–21.3 mg/kg bw/d)
Developmental neuro-toxicity study in mice (Eriksson et al., 2006)	0.9 and 13.5 mg/kg bw/d	Spontaneous motor behaviour, learning and memory processes affected.	0.9 mg/kg bw/d (LOAEL)
One-generation reproductive study in rats (Van der Ven et al., 2009)	0, 0.1, 0.3, 1.0, 3.0, 10.0, 30 or 100 mg/kg bw/d	Reduced bodyweights of F1 pups, delayed time to vaginal opening in female pups. Reduced weights of testis, kidney and prostate at lower doses in F1 males. Dose-dependent decreases in liver apolar retinoid in males and female pups.	BMD-L 82.2 mg/kg bw/d for vaginal opening and 94.6 mg/kg bw/d for reduced bodyweights of pups.
Two-generation reproductive study in rats (Ema et al., 2008)	0, 150, 1500 or 15 000 ppm	Dose-dependent pup mortality during lactation in the F2 generation. Decrease in bodyweights of male F1 and F2 pups and female F2 pups. Decreased weights of kidney, brain, spleen, adrenal, epididymis, ventral prostate and ovary at mid and high doses.	150 ppm for pup mortality (10.2 mg/kg bw/d)

Classification

The decrease in fertility indices noted in the 2-generation reproductive study was marginal and not statistically significant. The reduced number of primordial follicles noted in F1 females in the same study was within the range of values observed in animals of historical control groups evaluated in the same laboratory. These observations were therefore not considered significant enough to warrant classification of HBCD as toxic to fertility.

In the 1-generation reproductive study, decreased weights of testis and prostate in male and delayed vaginal opening in female weanlings were noted. In addition, increased pup mortality in the F2 generation and delayed physical development in F2 pups, such as delayed eye opening and effects on basic reflexes, were noted in the 2-generation study. Another study reported developmental neurotoxicity in pups of HBCD-treated rats. Based on these observations, HBCD meets the Approved Criteria for classification as toxic to reproduction, category 3, with the risk phrase R63, "Possible risk of harm to the unborn child".

Effect through lactation

According to the Approved Criteria, substances which cause concern due to toxicity when transferred to the baby during the period of lactation should be classified as "R64 – May cause harm to the breastfed babies".

It is quite likely that the demonstrated alterations in postnatal development observed in 1- and 2-generation studies of Ema et al. (2008); Saegusa et al. (2009) and Van der Ven et al. (2009) were due to exposure to HBCD through milk during lactation. This is particularly evident in the Ema et al. study, where pup mortality occurred at day 4 postnatally (not seen on day 0) and markedly increased till day 20. There was also statistically significant reduction in bodyweights of F2 pups during the lactation period, with highest reduction on day 21 of lactation (before weaning began), providing evidence that exposure via lactation was responsible for increased mortality and decrease in bodyweight.

Numerous human monitoring studies have shown that HBCD is present in human breast milk. A recent monitoring study by Eljarrat et al. (2009) reported significantly higher levels of HBCD levels (up to 188 ng/g lw) in Spanish human milk as compared to those reported earlier. The authors attribute the increase in the reported HBCD levels in human milk to increased usage of (and exposure to) HBCD over time.

HBCD is a very lipophilic compound, which is persistent and tends to accumulate in the fat in many species, including man. This indicates that the substance has the potential to accumulate to toxic levels in breast milk. In general, newborns are more vulnerable to toxic effects. Taken together, these findings indicate a concern for the health of breastfed children.

Based on pup mortality in rats during the lactation period and the occurrence of HBCD in human breast milk reported in populations from various countries, and the fact that HBCD is known to persist and accumulate in organisms, HBCD is assigned the risk phrase R64 (May cause harm to breastfed babies).

The classification of HBCD under the Globally Harmonised System of Classification and Labelling of Chemicals is provided in Appendix I.

9. Environmental hazard assessment

The assessment of the environmental fate of HBCD concluded that it was persistent in the environment and very bioaccumulative. These properties make results of traditional ecotoxicity tests difficult to interpret. The effects associated with such persistence and accumulation of the compound are unpredictable in the longer term and standard toxicity tests cannot be performed for long enough durations to assess their potential effects over long periods. Therefore, results obtained from data reviewed in the following chapter should be treated with a degree of caution.

Commercial HBCD is comprised of 3 isomers, each with differing water solubility (see Chapter 3). Briefly, α-HBCD is in the order of 20 times more soluble than the γ isomer while β-HBCD is around 6 times more soluble than the γ isomer. Nonetheless, all isomers are poorly soluble in water, and the composite compound has a correspondingly high LogKow of around 5.6. The poorly soluble nature of the substance may influence how ecotoxicity tests are conducted and interpreted.

Test data for effects of HBCD are available for fish, aquatic invertebrates, algae, sediment organisms, earthworms and 6 plant species in the form of a seedling emergence study. Table 9.1 summarises the results.

Table 9.1. Summary of ecotoxicity data for HBCD

		Result [µg/L measured]		
Test species	**Test system**	**LC50**	**NOEC**	**Reference**
Fish				
Rainbow trout (*O. mykiss)*	96 h flow through	–	2.5	Graves & Swigert, 1997a
Rainbow trout (*O. mykiss)*	88 d flow through		3.7	Drottar et al., 2001
Aquatic invertebrates				
Daphnia magna	48 h flow through	–	3.2	Graves & Swigert, 1997b
Daphnia magna	21 d flow through		3.1 MATC = 4.2	Drottar & Krueger, 1998
Algae / aquatic plants				
Green alga (*S. capricornutum)*	96 h	-	3.7	Roberts & Swigert, 1997
Marine diatom (*S. costatum*)	72 h (limit test)	>41	<41	Desjardins et al., 2004

		Result [µg/L measured]		
Test species	**Test system**	**LC50**	**NOEC**	**Reference**
Marine alga (*Chlorella* sp.)	72 h	>1500	-nr[1]	Walsh et al., 1987
Marine alga (*T. pseudonana*)	72 h	50–380	-nr	Walsh et al., 1987
Marine alga (*S. costatum*)	72 h	9.0–12.2	-nr	Walsh et al., 1987
Microorganisms				
Activated sludge	3 h	29% inhibition @ 15 mg/L mg/kg dw		Schaefer & Siddiqui, 2003
Sediment dwelling organisms				
Amphipod (*H. azteca*)	28 d, exposure through sediment	–	1000	Thomas et al., 2003a Thomas et al., 2003b
Terrestrial plants				
Six terrestrial plant species	Seedling emergence		6200	Porch et al., 2002
Earthworms				
Earthworm (*E. Fetida*)	56 d reproduction	771	21.6[2]	Aufderheide et al., 2003

1) NOECs not reported; 2) EC_{10} – Extrapolated value.

9.1 Avian toxicity

No standard test data are available.

HBCD was implicated as being partially responsible for delayed egg laying and smaller eggs being laid, causing thinner eggshells and differential weight loss during embryonic development, and reduced fertility and reproductive success in captive American ketstrels (*Falco sparverius*) (Fernie et al., 2009). However, the conclusions need to be treated with caution, as HBCD was not the focus of this study and conclusions were drawn essentially on a single, unintentional HBCD concentration (noting the "low" dose was not statistically different to control levels), and HBCD was present along with a large number of other polybrominated diphenyl ether congeners that were the original focus of the study.

9.2 Aquatic toxicity

HBCD has a low acute toxicity to aquatic organisms owing in part to its limited solubility in aqueous media. However, long-term exposure to HBCD appears to cause high toxicity in aquatic organisms, as seen in *Daphnia magna* and *Skeletonema costatum*. In both tests calculated NOEC and EC_{50} values were below the water solubility of the technical mixture of HBCD.

9.2.1 Toxicity to fish

Acute toxicity

Graves and Swigert (1997a) tested the acute toxicity to rainbow trout (*Oncorhynchus mykiss*) in a 96 h flow-through system and test concentrations of 1.5 to 6.8 µg/L. The test was based on OECD TG 203 and was performed to GLP. No mortality or sublethal effects were seen in any fish at any of the exposure concentrations throughout the test. Based on this test, HBCD appears to have no effect on rainbow trout up to its limit of water solubility.

Subchronic and chronic toxicity

Drottar et al. (2001) evaluated the toxicity of HBCD during early life-stage development of rainbow trout (*Oncorhynchus mykiss*). Exposure concentrations ranged from 0.63 to 6.8 µg/L. Hatching success, time to hatch, time for larvae to swim up and post-hatch growth and survival were evaluated during the 88 d test. The study was based on OECD TG 210 and OPPTS 850.1400 and followed GLP. There was no statistically significant difference in the growth measurements (length and wet weight) between control groups and treatment groups. All fish appeared normal and healthy throughout the study period. From this test, HBCD was not chronically toxic to rainbow trout in their early life stage up to the level of solubility. The NOEC was determined to be 3.7 µg/L.

Effects of HBCD on biomarkers in juvenile rainbow trout (*Oncorhynchus mykiss*) were studied by Ronisz et al. (2004). Fish were injected once intraperitoneally with HBCD at 50 or 500 mg/kg dissolved in peanut oil, although the high concentration dose was not possibly fully dissolved in peanut oil. The experiments lasted for 5 and 28 d. Bile analysis after the 28 d experiment showed that the concentration of HBCD in the bile was 0.14 to 0.7 µg/g for the 50 mg dose and 7 to 15 µg/g for the 500 mg dose. Different hepatic enzyme activities (catalase, glutathione S-transferase, glutathione reductase, and ethoxyresorufin-O-deethylase (EROD)), the liver somatic index, the occurrence of DNA-adducts in the liver, and plasma vitellogenin levels were measured. No consistent effects were observed after 5 d. After 28 d, a 40% increase in the liver somatic index was observed in the high-dose group. Also in this group, EROD activity was significantly inhibited (>80%).

Palace et al. (2008; 2010) studied the effects of environmentally relevant concentrations of HBCD diastereoisomers (α-, β- and γ-HBCD) on phase I and II biotransformation enzymes, cytochrome P4501A (CYP 1A) and glucuronyltransferase (UDPGT), regulation of thyroid status (circulating free T3 and T4), thyroid gland structure, T4 outer ring deiodinase (T4ORD) activity in rainbow trout (*Oncorhynchus mykiss*). These studies concluded that thyroid hormone metabolism is altered in organisms exposed to HBCD (details in Section 9.5).

In another study, Smolarz and Berger (2009) demonstrated the ability of HBCD to induce nuclear and nucleolar abnormalities and to increase cell death (via apoptosis and necrosis) in gills of benthic clams (*Macoma balthica*) exposed to nominal HBCD concentrations of 0, 100 and 250 µg/l over a period of 50 d. Main peaks for nuclear abnormalities and malfunction of ribosomal genes were observed after 10 and 20 to 30 d, respectively. According to the authors, the induction of micronuclei and other nuclear abnormalities reflect the genotoxic potential of HBCD to marine invertebrates.

Expression of 38 different proteins, many of which were related to protein metabolism, cellular defence and cytoskeleton dynamics was shown to be altered in livers of zebrafish (*Danio rerio*) exposed to 0.05, 0.5, 5 or 187 µM of a technical HBCD mixture for 24 and 72 h (Kling & Förlin, 2009).

9.2.2 Toxicity to aquatic invertebrates

Acute toxicity

The acute toxicity of HBCD to *Daphnia magna* was evaluated in a 48 h flow-through study performed according to OECD TG 202 and GLP (Graves & Swigert, 1997b). Exposure concentrations range from 1.5 to 6.8 µg/L. No mortalities or other signs of toxicity were observed in the negative and solvent control groups throughout the test. The same was observed in all treatment groups, with the exception of one mortality in the second highest treatment group. In this test, HBCD had no effect on daphnids up to its level of water solubility.

Chronic toxicity

Drottar and Krueger (1998) evaluated the chronic toxicity of HBCD on survival, growth and reproduction of *Daphnia magna* during a 21 d exposure period under flow-through conditions. The test was performed according to OECD TG 202 and GLP. Exposure concentrations ranged from 0.85 to 13.6 µg/L. No treatment-related mortality was observed. No statistically significant effects on survival, reproduction and growth were observed at 3.1 µg/L or less. Therefore, the 21 d NOEC was determined to be 3.1 µg/L, which is approximately the water solubility of HBCD. Daphnids exposed to 5.6 µg/L for 21 d had statistically significant reduced mean lengths. Thus the LOEC was determined to be 5.6 µg/L, with a resulting Maximum Acceptable Toxic Concentration (MATC) of 4.2 µg/L.

HBCD effects in a partial life cycle test with zebrafish (*Danio rerio*) were reported at the Society of Environmental Toxicology and Chemistry (SETAC), Europe, 2006 meeting (Kuiper et al., 2006). General and reproductive health in zebrafish was measured after exposure of parents (29 d) and subsequent exposure of eggs and juveniles (45 d) to a dilution series based on the maximum solubility by recirculating exposure water over spiked chromosorb. Adult and juvenile fish were chemically analysed for dose assessment. Reproduction was assessed by egg production, fertilisation rate, juvenile development (hatching, survival, length, weight and condition factor) and animals were examined histologically (general pathology, gonad development, thyroid morphology). HBCD levels in animals were well dictated by the nominal water concentrations (tested up to 165 µg/L). HBCD levels in females exceeded those in males, and males exceeded those in juveniles.

Consistent with contamination profiles in biota in the wild, α-HBCD dominated the isomeric profile in fish. Reproduction/adult histology results showed egg production

was highly variable. The frequency of successful mating decreased with increasing dose and was consistent with a histologically observed mild increase in ripe oocytes; however, this did not result in a dose-dependent decrease of cumulative egg production. Exposure-related effects on fertilisation and hatching rates were not observed. No dose-dependent morphologic effects were observed in other organs, including thyroids. Juvenile development results showed an increased frequency of animals with early yolk vesicle stage oocytes. Other effects on juvenile development including gender distribution and survival were not observed. This study also formed part of the EU's FIRE project (Appendix 4).

9.2.3 Toxicity to algae / aquatic plants

Roberts and Swigert (1997) evaluated toxicity of HBCD to the freshwater green alga (*Selenastrum capricornutum*) in a 96 h exposure study. The test was based on OECD TG 201 and was performed to GLP. Nominal exposure concentrations ranged from 1.5 to 6.8 µg/L. No dose response was found. Inhibition of around 10% based on area under curve (AUC) after 96 h was observed in the highest tested treatment. However, the findings are inadequate to calculate EC50 results. Averaging the measured concentrations at the start and the end of the test for the highest test group resulted in a mean exposure concentration of 3.7 µg/L (corrected value based on recovery). This must be considered the NOEC for the test, and approximates the measured water solubility of total HBCD.

Desjardins et al. (2004) determined the toxicity of water-soluble components of HBCD to the marine diatom (*Skeletonema costatum*) using saltwater algal media passed through a generator column saturated with HBCD. The test was based on OECD TG 201, ISO 10253:1995, EU Method C.3 and GLP. A single concentration was used and the arithmetic mean of total HBCD at test termination was 41.0 µg/L. Inhibition was observed based on both cell density and AUC. Based on cell density, inhibition was 19% and 31% compared with the control with no generator column and the control with the generator column, respectively. Based on AUC, inhibition was 21% and 31% compared with the control with no generator column and control with the generator column, respectively.

Walsh et al. (1987) studied the toxicity of HBCD to the marine unicellular algae *Skeletonema costatum, Thalassiosira pseudonana* and *Chlorella* sp. The tests were carried out for either 72 h (*S. costatum* and *T. pseudonana*) or 96 h (*Chlorella* sp.). Six different growth media were used in the test – one natural seawater and five synthetic seawater formulations (5 growth media tested with *S.costatum*). *Chlorella* sp. was not inhibited by as much as 50% at 1.5 mg HBCD/L. However, HBCD appeared toxic to *S. costatum* and *T. pseudonana* below the estimated saturation concentration. EC50 values were determined in 4 of the 5 test media for *S. costatum* and ranged from 9.0 to 12.2 µg/L. This species was more sensitive than *T. pseudonana*, where EC50 values were determined in all 6 test media and ranged from 50 to 370 µg/L. The lowest EC50 for this species approximates the maximum water solubility of HBCD in saltwater as determined above in Desjardins et al. (2004). However, the results for *S. costatum* were all below the apparent maximum water solubility of HBCD. With multiple data available for the same species with the same endpoint, the geometric mean value can be used. In this case, the geometric mean EC50 for this species was determined to be 10.5 µg/L. For this species, there was little variability between the results (standard deviation = 1.05), regardless of the test medium.

9.2.4 Sediment dwelling organisms

Thomas et al. (2003a) performed a prolonged sediment toxicity test with the amphipod *Hyalella azteca* using a flow-through test system with sediments of nominal 2% organic carbon content. The test protocol was based on the ASTM E 1706-95b Guideline and USEPA Series 850 Ecological Effects Test Guidelines (OPPTS No. 850.1735). Nominal test concentrations were prepared by weighing the test substance and adding directly to the dry sediment in a range of 31 to 1000 mg/kg dw sediment. The mean number of amphipods in the treatment groups was not statistically different ($p > 0.05$) from the control group. The dry weights in the treatment groups were not significantly different from the control weights. The 28 d EC50 for amphipods exposed to HBCD in sediment with 2.3% OC was >1000 mg/kg dw sediment, the highest rate tested. Based on the results of this study, the LOEC was >1000 mg/kg dw sediment and the NOEC was 1000 mg/kg dw sediment.

A second study following the same methodology and test concentrations was performed using a nominal 5% OC (actual, 4.7%) sediment (Thomas et al., 2003b). In this study, all replicates observed during the test appeared normal, with mortality significantly different from control replicates in the 63, 125 and 250 mg/kg treatment groups. However, given the lack of mortality in the higher treatment groups, these findings are not considered to be treatment-related. No statistically significant effects on growth (dry weight) were found at any treatment level. The 28 d EC50 for amphipods exposed to HBCD in sediment with 4.7% OC was >1000 mg/kg dw sediment, the highest rate tested. Based on the results of this study, the LOEC was >1000 mg/kg dw sediment and the NOEC was 1000 mg/kg dw sediment.

9.2.5 Toxicity to microorganisms

HBCD's effect on the respiration of activated sludge microorganisms was assessed using control, reference and treatment groups (Schaefer & Siddiqui, 2003). The test was performed to OECD TG 209 and GLP. The control group was used to determine the background respiration rate of the sludge and was not dosed with the test or reference substance. The reference group was dosed with 3,5-dichlorophenol, a known inhibitor of respiration, at nominal concentrations of 3, 15 and 50 mg/L. The test substance was dosed at a limit concentration of 15 mg/L. After an exposure period of around 3 h, the respiration rates of the test solutions were measured using a dissolved oxygen meter. The individual respiration rates of the 2 controls were 60.5 and 55.5 mg O_2/L/h. The difference between the 2 control respiration rates was 9.0% and was within the 15% difference limit established for the test. The validity of the test was further supported by the results from the 3,5-dichlorophenol reference group, which resulted in an EC50 of 5.2 mg/L and was within the 5 to 30 mg/L range considered acceptable for the test. An average of 29.1% inhibition was observed in the treatment group.

9.3 Terrestrial toxicity

9.3.1 Soil microorganisms

No data are available.

9.3.2 Plants

The toxicity of HBCD to 6 species of plants has been determined using OECD TG 208 (the protocol is based on the 1998 proposal for revision of this test guideline) and OPPTS 850.4100 and 850.4225 in a seedling emergence study (Porch et al., 2002). Nominal test concentrations ranged from 40 to 5000 mg/kg soil dw. There were no apparent effects on any endpoint for any of the 6 plant species tested. Statistical analyses indicated no significant differences between the control and treatment group mean emergence, survival, height or weight for corn, cucumber, ryegrass, soybean and tomato. Overall, the NOEC from these studies was ≥6200 mg total HBCD/kg dry soil based on the mean measured concentration in soil at the start of the test.

9.3.3 Earthworms

A reproduction test with earthworms (*Eisenia fetida*) was performed following OECD TG 207 and OPPTS 850.6200 (Aufderheide et al., 2003). Nominal test concentrations ranged from 78.5 to 5000 mg/kg dw soil. In tissue samples, α-HBCD dominated the isomer profile ranging from 51.8% to 73.1% of total HBCD found in tissues. γ-HBCD was next most abundant (13.8%–37.7% total HBCD) and β-HBCD was found at the lowest levels of 10.4% to 17% total HBCD.

No abnormal burrowing or avoidance behaviour was seen in the first 60 minutes of the test. The 28 d mortality NOEC was 4190 mg total HBCD/kg dw soil. The estimated EC_{10} and EC_{50} values for adult earthworm survival were >4190 mg total HBCD/kg dw soil. The 56 d reproduction NOEC was 128 mg total HBCD/kg dw soil. At the lowest rate tested, there was still a 15% reduction on reproduction. Consequently, the reproduction NOEC value was estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test. The reproductive data were shown to satisfy the assumptions of normality and homogeneity of variance and the ANOVA was performed on the raw data. The estimated EC_{10} and EC_{50} values for average reproduction were 21.6 mg/kg dw soil (95% CI 0.000468–110 mg/kg dw soil) and 771 mg/kg dw soil (95% CI 225–4900 mg/kg dw soil) respectively.

9.4 Toxicity via the atmosphere

No test data on animals exposed through the gas phase are available, and only abiotic effects of HBCD will be considered in this section.

Very low concentrations of HBCD are predicted for the atmospheric compartment. Removal is likely to be mainly via wet and dry deposition, although photodegradation may also occur to some extent. Thus, HBCD can be considered to present a negligible risk of adding to effects such as global warming, ozone depletion in the stratosphere and acidification.

9.5 Toxicity through endocrine disruption

As part of a project to assess the risk of brominated flame retardants as suspected endocrine disruptors for human and wildlife health (FIRE project), studies were conducted in rats and aquatic organisms to determine the effects of HBCD on some endocrine parameters. The studies in rainbow trout (*Oncorhynchus mykiss*) and European flounder (*Platichthys flesus*) are summarised below (see Appendix 4 for full details of the project).

Palace et al. (2008) examined the hypothesis that environmentally relevant concentrations of HBCD diastereoisomers may affect biotransformation enzymes (cytochrome P4501A (CYP 1A) and glucuronyltransferase (UDPGT)) and the regulation of thyroid status (circulating free T3 and T4, thyroid gland structure and T4 outer ring deiodinase enzyme, T4ORD activity) in rainbow trout (*Oncorhynchus mykiss*).

Four formulations of food were prepared, 3 of which were spiked with a known amount of a particular diastereoisomer, along with a control formulation. Lipid corrected mean concentrations of α, β and γ isomer in the food were determined to be 29.14, 11.84 and 22.84 ng/g, respectively. Juvenile rainbow trout were separated into 4 800 L fiberglass tanks (n = 50 in each tank) receiving water at a constant water flow of 1.5 L/min. Fish in each tank were exposed to an individual HBCD isomer via their food for an uptake phase of 56 d, followed by a depuration phase of 112 d. Four fish from each tank were sacrificed on days 0, 7, 14, and 56 of the uptake phase and days 7, 14, 56, and 112 of the depuration phase for analysis.

Free triiodothyronine (T3) and thyroxine (T4) were determined on days 0, 7, 14, and 56 of the uptake phase and day 112 of the depuration phase. T4 outer ring deiodinase enzyme activity (T4ORD) was determined in liver microsomes from days 14 and 56 of the uptake phase of the experiment. Analyses of phase I enzyme activity, as ethoxyresorufin-O-deethylase (EROD), and of the phase II enzyme UDPGT were performed in microsomes prepared from livers of exposed and control fish.

No mortalities occurred during the experiment and no significant changes were seen in the growth parameters of the fish. Differences in plasma thyroid hormone levels were noted in all experimental groups. Thyroid hormone concentrations are influenced by reproductive state, age and nutritional status and varied among all treatment groups between sampling periods. The differences between treatment groups at each sampling point indicate that the thyroid system is affected in fish exposed to HBCD. This was evident in the concentrations of both T4 and T3 in blood plasma. Disruption of the thyroid axis was most evident in the γ-HBCD exposed group, as indicated by lower circulatory FT4 and higher FT3 as well as an increase in thyroid epithelial cell height. There were significantly higher concentrations of T3 in fish exposed to the γ isomer at day 56 of the uptake phase and day 14 of the depuration phase and in fish exposed to the β isomer after 56 d of depuration, relative to the reference. There was also lower T4 in α, β, and γ exposed fish at day 56 uptake and days 7 and 56 of depuration in the γ exposed fish.

All HBCD-exposed fish groups sampled on day 7, and the fish fed the α and β isomers sampled on day 56, had lower EROD activity than the fish fed the reference diet. No other significant induction of EROD was evident in any of the HBCD-treated fish. Glucuronylstransferase activity was elevated in liver of fish fed the α and β diets, but not in the γ group, at day 56 relative to the fish fed the reference diet. Although no differences were evident at day 7 of the clearance phase, by day 14, fish fed the β and γ diet had significantly lower activity than the reference fish. This difference persisted in the γ group to day 112 of the clearance phase.

T4ORD was assessed at day 14 of the uptake phase, and also when maximal HBCD tissue concentrations were achieved (day 56 of the uptake phase). This latter time coincided with the period when T3, T4 and thyroid glandular structure were altered. T4ORD activity was similar among all groups after 14 d of uptake but was significantly lower among all HBCD-exposed fish compared to the reference fish after 56 d of the

uptake phase of the experiment. The authors suggest that higher concentrations of circulating T3 in the HBCD-exposed fish may have contributed to the lower T4ORD enzyme activity, as high T3 concentrations have been shown to negatively feedback to lower T4ORD by decreasing the amount of enzyme protein in liver cells.

Thyroid epithelial cell heights were significantly greater in the γ-HBCD-exposed group at day 56 of the uptake phase and in fish from the α- and γ-HBCD exposed groups at day 14 of the depuration phase. Enlarged thyroid epithelial cells are a reliable measure of thyroid gland hypertrophy and in rats are one of the most sensitive effects of HBCD exposure. In summary, environmentally relevant concentrations of α and γ isomers of HBCD, and similar concentrations of β-HBCD, in the diet altered the thyroid status of juvenile rainbow trout. Lower circulating concentrations of T4 and higher T3 as well as altered hepatic metabolic enzymes and thyroid gland hypertrophy were evident. Disruption of the thyroid axis was transient and alterations to circulating hormone concentrations, conjugation enzymes and thyroid glandular structure were most evident when HBCD concentrations were highest in fish from this experiment.

In a more recent study, Palace et al. (2010) showed that, in juvenile rainbow trout, the tissue disposition and elimination rates of orally administered T4 hormone [$^{(125)}$]-T4] were altered by HBCD. In particular, on day 14 after gavage feeding, there was significantly lower radioactivity in the thyroid of fish exposed to the HBCD isomers relative to the reference group. This implies that either there was lower iodide uptake by the gland in fish exposed to HBCD or that the rate of thyroid hormone turnover in HBCD-treated fish was elevated. There was also a significantly higher type II outer ring deiodinase enzyme activity in livers of fish exposed to the beta and gamma isomers. Taken together, these results indicate that HBCD can potentially impact the thyroid system of fish.

Effects of HBCD on European flounder (*Platichthys flesus*) were studied by Kuiper et al. (2007). European flounder were chronically exposed to HBCD (78 d) in a wide range of concentrations, including environmentally relevant concentrations. HBCD was administered in food and sediment, or in sediment alone. Chemical analysis of muscle showed an average increase in internal concentrations of approximately 2 orders of magnitude. Animals exposed to HBCD via sediment alone (8000 µg/g total organic carbon, TOC) showed a proportional increase of α-HBCD in muscle compared to animals exposed via food and sediment. Exposure to the test compound did not affect general health and toxicity parameters (behaviour, survival, growth rate and relative liver and gonad weight). Hepatic microsomal enzyme activities (EROD, PROD and BROD) were not induced by any of the tested chemicals. Aromatase activity in male gonads showed a mild increase with rising TBBPA levels. There were no morphological and immunohistochemical indications for increased production of the yolk precursor protein vitellogenin (VTG) in animals. Other organs investigated (liver, gills, kidney, skin and gonads) revealed no histological changes related to HBCD exposure.

Overall, the present results indicate limited endocrine effects of this widely-used flame retardant in a test species representative of European estuaries at environmentally relevant exposure levels and at internal levels up to 446 µg HBCD/g lipid weight in flounder muscle.

9.6 PNEC derivation

Calculating "safe" concentrations for compounds such as HBCD that are persistent and have been found to be very bioaccumulative and biomagnify in the food chain is

difficult. This is because, while persistence and bioaccumulation are not adverse effects, potential adverse effects from exposure to such chemicals may not manifest themselves for very long periods – much longer than can be captured by standard toxicity testing. In this regard, it is noted that in some international jurisdictions – for example, Canada – long-term effects are assumed for substances that are considered very persistent and very bioaccumulative.

The relevance of the values calculated in this section will be considered more fully in the risk characterisation.

9.6.1 $PNEC_{aquatic}$

There are acute and chronic toxicity data available for 3 trophic levels. Often, EC/LC50 values were not definable. Several tests measured endpoints in terms of total HBCD and, based on solubility findings, it appears that, for all trophic levels, the NOEC for HBCD is at or around the measured total HBCD solubility of 3.4 µg/L. However, other solubility testing where individual stereoisomers were considered suggests that total solubility may be somewhat higher than this level, with α-HBCD being some 20 times more soluble than γ-HBCD.

The most sensitive defined acute EC50 was for the marine diatom *S. costatum*, with a geometric mean EC50 of 10.5 µg/L. While this is from an older study and reflects total HBCD, where the test substance was dissolved in a solvent prior to testing, it is still within the solubility range determined for HBCD (around 41 µg/L) when testing with all 3 isomers.

Chronic data are also available for the 3 trophic levels. Based on these data, the most sensitive species was *Daphnia magna*, with a NOEC of 3.1 µg/L and a MATC of 4.2 µg/L. Because chronic NOECs are available from 3 species representing 3 trophic levels, an assessment factor of 10 is applied to the lowest NOEC.

This results in a $PNEC_{aquatic}$ of 0.31 µg/L.

As noted above, a true PNEC is difficult, if not impossible, to derive for persistent and bioaccumulative substances such as HBCD. For higher trophic levels, food consumption is likely to be the primary route of exposure for such substances in the field. Therefore, the PNEC could underestimate effects if this pathway is not considered, especially in top predators.

9.6.2 $PNEC_{sediment}$

Only 2 laboratory reports were available for assessment of effects on sediment-dwelling invertebrates. However, these were treated as one study because the tests were identical, with the exception that the sediments in each test had different levels of organic carbon. In both, the test material was applied to the sediment, not through the water column, as experience has shown that this can often lead to more sensitive results.

The results showed a NOEC to the amphipod (*H. azteca*) of 1000 mg/kg dw sediment, the highest tested rate. Evidence from these test reports suggests that the amphipod was the most sensitive of 3 different sediment organisms considered in a non-GLP screening test undertaken to determine which organism to study in the definitive test. However, again all exposure was through sediment that was spiked while dry.

While the tests were prolonged (28 d), they did not allow for determination of effects of a more chronic nature such as reproduction; rather, they focused on survival and growth. For this reason, they are considered short-term and, with only one study available, an assessment factor of 1000 is used.

Using this assessment factor approach, a $PNEC_{sediment}$ of 1 mg/kg dw is determined.

Given the limited data relating to sediment dwelling organisms, and considering sediments are likely a significant sink for HBCD in the environment, a $PNEC_{sediment}$ can also be derived using equilibrium partitioning methodology as described in the EU TGD (EC, 2003) with relevant default values taken from the Level III fugacity model. These default values include a fraction of organic carbon in suspended matter (Foc_{susp}) of 0.2, sediment composition of 20% solids and 80% water, a bulk density of solids of 2400 kg/m^3 and, therefore, a density of sediments of 1280 kg/m^3.

The first step to estimating the $PNEC_{sediment}$ is to determine the partition coefficient for solid–water in suspended matter. This is the Foc_{susp} x Koc and results in a Kp_{susp} = 23,520 L/kg. This value represents the concentration of the substance sorbed to solids (mg/kg) divided by the concentration dissolved in pore water (mg/L).

The next step is to convert this value to the whole sediment compartment. In sediment, solids are assumed to account for 20% of the sediment compartment. Therefore, the sediment whole compartment Kp must be converted to account for the make-up of the sediment (80% water, density 1000 kg/m^3; and 20% solids, density 2400 kg/m^3). The whole compartment partition coefficient is the concentration in solids (mg/m^3) divided by the concentration in water (mg/m^3).

Therefore, the $K_{susp\text{-}water}$ is calculated as $0.8 + 0.2 \times Kp_{susp}/1000 \times 2400 = 11\ 290$ m^3/m^3.

The final step is to convert the PEC_{water} to a $PEC_{sediment}$ based on the $K_{susp\text{-}water}$ partition coefficient, and the density of the sediment compartment (1280 kg/m^3). This is achieved as follows:

$PEC_{sediment} = K_{susp\text{-}water}/Density_{sed} \times 1000 \times PNEC_{water}$

This results in a $PNEC_{sediment}$ of 2.7 mg/kg ww.

The EU TGD recommends for compounds with a LogKow >5 that, in order to take uptake via ingestion of sediment into account, the PEC/PNEC ratio is increased by a factor of 10. Therefore, the $PNEC_{sediment}$ becomes 0.27 mg/kg ww. Based on the default sediment characteristics above, this converts to a $PNEC_{sediment}$ of 0.72 mg/kg dw.

The 2 $PNEC_{sediment}$ values derived through either the assessment factor method or the equilibrium partitioning approach are consistent. However, there remains considerable uncertainty about this level due to the lack of testing on sediment organisms.

9.6.3 $PNEC_{soil}$

Test data are available for plants (seedling emergence study only) and earthworms (56 d reproduction test). No effects due to HBCD exposure were found on seedling emergence for 6 different terrestrial plant species up to a measured soil level of 6200 mg/kg total HBCD. In the earthworm study, based on reproductive effects, an EC50 of 771 mg/kg soil was derived. A NOEC was established at 128 mg/kg due to effects at this level and below not being considered statistically significantly different to the control. However, 15% inhibition of reproduction compared with the controls was still observed at the lowest tested mean measured concentration of 51.5 mg/kg. An extrapolated EC10 of 21.6 mg/kg was calculated for this study.

There are 2 NOECs available for 2 trophic levels – namely, primary producers (e.g. algae) and primary consumers (e.g. herbivores). Adopting the approach that the same assessment factors applicable to an aquatic toxicity dataset can be applied when determining a $PNEC_{soil}$, an assessment factor of 100 could be used based on available OECD guidance. However, in this case, because the most sensitive result came from a chronic earthworm study, an assessment factor of 50 will be used based on the EU TGD.

Applying this factor, a $PNEC_{soil}$ of 0.43 mg/kg dw is derived.

9.7 "Persistent, bioaccumulative and toxic" assessment of HBCD

The PBT assessment is considered separately to the deterministic risk assessment approach because it may prove more difficult to estimate risks for a chemical that is classed as PBT.

Traditional risk assessment methodologies may not adequately address concerns related to PBT chemicals for various reasons. Such substances may accumulate in parts of the environment and such accumulation could be very difficult to reverse. Current levels found in the environment may not reflect future maximum concentrations, as such persistent substances may take very long periods of time to reach equilibrium. Also, the available data on ecotoxicological effects may be inadequate, as effects associated with such accumulation are unpredictable in the longer term and standard toxicity tests cannot be performed for a long enough duration for potential effects to manifest themselves. There is also an additional consideration that remote and pristine environments should be protected and remain free of hazardous substances resulting from anthropogenic activity.

Once these substances enter the wider environment, any cessation of emissions will not necessarily result in a reduction in chemical concentration and hence any effects become difficult to reverse. Also, because of the long-term exposures and long life cycle of many important marine species, effects may be difficult to detect at an early stage.

While persistence and bioaccumulation themselves are not adverse effects, the consequence of the latter point above is that a "safe" concentration is difficult if not impossible to establish.

In undertaking a PBT assessment, it is first necessary to identify PBT substances using specific criteria for the inherent properties of the chemical. Australian PBT criteria have been considered in this assessment (EPHC, 2009). The Stockholm Convention criteria are in the context of POPs; therefore, they can be defined as being for very persistent and very bioaccumulative substances. A chemical deemed persistent or bioaccumulative by Australian criteria may not carry values as high as those prescribed in the POPs criteria.

9.7.1 PBT criteria

1) Persistence criteria

The Stockholm Convention (to which Australia is a party) provides scientifically-based criteria for potential POPs and a process that ultimately may lead to elimination of a POP substance globally. The criteria for persistence in Annex D of the Convention are expressed as single-media criteria as follows:

Evidence that the half-life of the chemical in water is greater than 2 months, or that its half-life in soil is greater than 6 months, or that its half-life in sediment is greater than 6 months, or

Evidence that the chemical is otherwise sufficiently persistent to justify its consideration within the scope of the Convention.

The following persistence criteria have been adopted in Australia for persistent chemicals, with definitions from the Stockholm Convention remaining for very persistent compounds (EPHC, 2009).

Media	**Half-life**
Water	>2 months
Soil	>6 months
Sediment	>6 months
Air	>2 days

2) Persistence of HBCD

Considerable analysis of persistence of HBCD in various environmental media has been undertaken.

To model the environmental fate of chemicals, single media half-lives are required. Several test results have been described above, and these along with modelled information will be used to determine half-lives used for multimedia modelling purposes.

i) Air

HBCD has a low vapour pressure and, if present in the atmosphere, will most likely be sorbed to aerosol particles to a considerable degree. For such chemicals, the OH rate constant is difficult to measure and no data are available for HBCD

The half-life of HBCD in the atmosphere through reaction with hydroxyl radicals was calculated using the AOPWIN Program, resulting in a predicted half-life based on a 12 h light day, 2.13 d, or 51.1 h.

ii) Water

HBCD is expected to be hydrolytically stable in water. The chemical is not ready biodegradable. This result can be used to predict a water half-life. The EU default for a non ready-biodegradable chemical is 1 000 000 d (EC, 2003) while the US EPA use 10 000 d or other appropriate default for no biodegradation (US EPA – OPPT). Negligible degradation in water will be assumed for modelling in this assessment.

iii) Soil

HBCD is not ready biodegradable and was shown to not be inherently biodegradable under aerobic conditions in experiments.

One soil degradation test showed HBCD to degrade in aerobic soil when amended with activated sludge. The half-life was in the order of 63 d. In the non-amended soil little degradation was observed after 112 d. EPIWIN calculates a soil half-life of 1440 h, or 60 d, which is very similar to this tested level. However, this is not considered representative of a soil half-life where HBCD reaches the soil compartment other than as part of sewage sludge amendment.

A separate study investigating aerobic degradation in unamended soil showed HBCD to be resistant to biodegradation with no significant loss of test material (~10% total HBCD) over the 112 d test period. This is considered to be representative for HBCD exposure to soil other than through application in sewage sludge, and the half-life in soil will be treated as negligible for modelling in this assessment.

iv) Sediment

Some laboratory data are available showing apparent primary degradation of HBCD in soils and sediment under both aerobic and anaerobic conditions. While in cases this degradation appeared relatively fast (for example, half-lives of 11 to 32 d in aerobic water/sediment systems with corresponding half-lives of 1 to 1.5 d in anaerobic water/sediment systems), it was difficult to track degradation (the studies did not use ^{14}C-HBCD, recoveries were low, and low concentrations were tested that really only enabled an assessment of γ-HBCD), and these results are not considered valid for basing an assessment of persistence on. More recent studies using radiolabelled HBCD provided more insight. These studies used higher concentrations, enabling some consideration of the α- and β-HBCD isomers. Primary degradation half-lives were around 117 d and 73.5 d (total HBCD) under aerobic and anaerobic conditions respectively at around 20 ^{o}C in water/sediment systems. Laboratory evidence suggests HBCD will degrade faster under anaerobic conditions, and the conditions of this test are more indicative of reducing conditions than aerobic conditions. Therefore, this result may underestimate persistence in aerobic water/sediment systems. In both systems, there was no appreciable formation of $^{14}CO_2$, indicating mineralisation was not occurring. Three transformation products were formed and identified as tetrabromocyclododecene, dibromocyclododecadiene and, finally, cyclodecatriene, which is itself a known persistent product. There was evidence in these tests that α-HBCD, in particular, was more resistant to primary degradation than γ-HBCD.

EPIWIN predicts a sediment half-life of 5760 h or 240 d. This is around twice the half-life found in the experimental test system.

While the laboratory data do indicate HBCD will degrade in sediments, monitoring data suggest this process is not quick. Due to uncertainties between laboratory-generated degradation data and measured levels in the field indicating longer persistence, the modelled half-life value of 240 d in sediment will be used in the fugacity modelling.

There are large uncertainties surrounding the fate of HBCD in the environment. While laboratory data support a conclusion that the substance degrades faster under anaerobic conditions than aerobic, the mechanisms for this are unclear, as these studies often showed very fast abiotic degradation rates under anaerobic conditions with (often) negligible abiotic degradation under aerobic conditions. Further, monitoring data from sediments in the environment (where conditions are most likely to be anaerobic) show a wide range of levels, although limited time trend data show HBCD levels in sediments that do not support a conclusion for degradation of HBCD under anaerobic conditions.

Given these results, supported by available monitoring data, particularly for sediments, HBCD is considered to be very persistent in the environment based on POPs criteria, particularly part ii described above.

3) Bioaccumulation criteria

The criteria for bioaccumulation in Annex D of the Stockholm Convention are given as follows:

i) evidence that the BCF or BAF in aquatic species for the chemical is greater than 5000 or, in the absence of such data, that the LogKow is greater than 5

ii) evidence that a chemical presents other reasons for concern, such as high bioaccumulation in other species, high toxicity or ecotoxicity, or

iii) monitoring data in biota indicating that the bioaccumulation potential of the chemical is sufficient to justify its consideration within the scope of the Convention.

The Australian criteria for a substance to be considered bioaccumulative is a BCF or BAF >2000 or, in the absence of any BCF/BAF measurements, a LogKow >4.2 (EPHC, 2009).

4) Bioaccumulation of HBCD

BCFs (total HBCD) in whole fish of 8800 to 13 000 were determined experimentally in rainbow trout. These BCFs may even be an underestimation, as steady state was not apparent in the low-dose group after 35 d of uptake. Further, steady state did appear to be reached, and levels continued accumulating in the test fish well into depuration period with levels peaking 10 d after exposure ceased. Modelling predictions based on the results of this test suggested higher BCFs – 16 500 to 22,000 – for whole fish. In addition, the chemical was shown to persist in the fish with slow depuration half-lives of 19 to 30 d (up to 101 d for the DT90).

In addition, HBCD levels in biota supports a conclusion that the substance bioaccumulates and biomagnifies through the food chain. The isomer profile in organisms is somewhat different from that found in the commercial mixture and generally in sediments where the γ isomer dominates. In organisms, particularly higher up the food chain, the evidence points to an enrichment of the α isomer, with this dominating the isomer profile in many cases.

It is concluded that HBCD is very bioaccumulative based on the criteria described above.

5) Toxicity criteria

The criteria for toxicity in Annex D of the Stockholm Convention do not consist of numerical values but are given as follows:

(e) Adverse effects:

(i) Evidence of adverse effects to human health or to the environment that justifies consideration of the chemical within the scope of this Convention; or

(ii) Toxicity or ecotoxicity data that indicate the potential for damage to human health or to the environment.

In Australia, for PBT purposes, in respect of aquatic toxicity, a chemical may be considered toxic under the following circumstances (corresponding to criteria for GHS chronic category 1; EPHC, 2009):

Non-rapidly degradable substances for which there are adequate chronic toxicity data available	Chronic NOEC or EC_x (for fish)	≤0.1 mg/L and/or
	Chronic NOEC or EC_x (for crustacea)	≤0.1 mg/L and/or
	Chronic NOEC or EC_x (for algae or other aquatic plants)	≤0.1 mg/L
Rapidly degradable substances for which there are adequate chronic toxicity data available	Chronic NOEC or EC_x (for fish)	≤0.01 mg/L and/or
	Chronic NOEC or EC_x (for crustacea)	≤0.01 mg/L and/or
	Chronic NOEC or EC_x (for algae or other aquatic plants)	≤0.01 mg/L
Substances for which adequate chronic toxicity data are not available (providing criteria for P and B are met)	96 h LC_{50} (for fish)	≤1 mg/L and/or
	48 h EC_{50} (for crustacea)	≤1 mg/L and/or
	72 or 96 h ErC_{50} (for algae or other aquatic plants)	≤1 mg/L
	and the substance is not rapidly degradable and/or the experimentally determined BCF is ≥500 (or, if absent, the Log K_{ow} ≥ 4).	
Toxicity to other (terrestrial) organisms	Should be considered on a case-by-case basis, compared with the highly toxic classifications developed for agvet chemicals.	
Long-term toxicity or evidence such as endocrine disruption effects	Should be considered on a case-by-case basis.	

6) Toxicity of HBCD

For aquatic organisms, there are acute and chronic toxicity data available for 3 trophic levels. The most sensitive defined acute EC50 was for the marine diatom *S. costatum*, with a geometric mean EC50 of 10.5 μg/L. While this is from an older study and reflects total HBCD with the test substance dissolved in a solvent prior to testing, it is still within the solubility range determined for HBCD (around 41 μg/L) when testing with all 3 isomers.

For chronic test data, the most sensitive species was *Daphnia magna*, with a NOEC of 3.1 μg/L and a MATC of 4.2 μg/L.

Presently, evidence of long-term toxicity, such as endocrine-disrupting effects, is limited. However, based on the results of the standard toxicity testing, HBCD meets the Australian toxicity criterion for a non-rapidly degradable substance with adequate chronic toxicity data showing a chronic NOEC to *Daphnia* <0.1 mg/L. This is consistent with the EU, US EPA (high concern based on PBT Profiler criteria), and Canada.

7) Potential for long-range environmental transport

The above PBT assessment for HBCD has concluded the substance is not only PBT according to Australian criteria but also meets the Stockholm Convention criteria for these 3 characteristics. While consideration of long-range environmental transport (LRT) is not part of a PBT assessment, given the PBT characteristics of this chemical, it is worth considering briefly here as LRT potential is considered in POPs screening criteria.

The potential for LRT should be assessed from:

- measured levels in locations distant from the source
- monitoring data indicating LRT has occurred, or
- fate properties and/or model results demonstrating the potential for LRT/half-life in air greater than 2 d.

LRT potential is not an intrinsic property of a chemical pollutant; rather, it derives from both chemical properties (hence will only be a characteristic of certain chemicals) and environmental conditions.

In an assessment of the long-range transport potential of HBCD using several multimedia transport models, Wania (2003) concluded that HBCD had a low potential to reach remote areas, which is dependent on the long-range transport behaviour of the atmospheric particulate matter to which it sorbs. The characteristic travel distance (CTD) for HBCD from 2 models, TaPL3 and ELPOS, were comparable at 760 and 784 km respectively. Absolute values derived from these models should be treated with some caution. However, it is useful to compare outcomes with other chemicals in terms of ranking. Wania and Dugani (2003) also assessed the LRT of other PBDEs and PCBs using the same models. The results showed that the CTD of HBCD was comparable to those of other PBDEs ranging from tetraBDE to decaBDE, and much lower than the low to medium chlorinated PCBs.

Besides transport through the atmospheric route, long-range transport of chemicals can also occur as a result of other mechanisms such as ocean currents and biota. Despite modelling of atmospheric transport indicating a low potential to reach remote areas, the ability for HBCD to undergo long-range transport in the environment is supported by monitoring data (see below). HBCD has been found in air samples in northern Sweden and northern Finland.

A subcommittee of the Arctic Council, the Arctic Council Action Plan Steering Committee, reported that, based on monitoring levels in air and marine animals, while atmospheric measurements are limited, there is evidence for long-range atmospheric transport of HBCD to the Arctic.[6] The Arctic Council is a high-level intergovernmental forum that provides a mechanism to address the common concerns and challenges faced by the Arctic governments and the people of the Arctic.

At the fourth meeting of the POPs Review Committee, the contact group on HBCD concluded that HBCD is subject to long-range environmental transport based on evidence from modelling and measured data from remote areas (UNEP, 2008).

9.7.2 Conclusions

The PBT characteristics of HBCD have been compared to Australian PBT criteria and POPs criteria described in the Stockholm Convention.

The available test data on persistence provide variable results and there are uncertainties surrounding the behaviour of HBCD in the environment. However, based on laboratory data and international environmental monitoring data, there is sufficient evidence to conclude that HBCD will persist in the environment and meets both Australian and POPs criteria for persistence.

Data provided through both laboratory testing and environmental sampling of biota show the chemical (particularly the α isomer) is highly bioaccumulative and can biomagnify through the food chain. HBCD meets both Australian and POPs criteria for bioaccumulation.

Based on standard toxicity testing, HBCD meets international criteria for toxicity. While numerical values for toxicity are not included in the POPs criteria, there is sufficient evidence from test data to indicate that adverse effects on environmental organisms can occur at low concentrations. Australian toxicity criteria are met for toxicity.

[6] http://acap.arctic-council.org/_documents/BFR%20DRAFT%20fact%20sheet-Oct1-2004.pdf#search=%22brominated%20flame%20retardants%20in%20the%20arctic%22

10. Human health risk characterisation

A Margin of Exposure (MOE) methodology is used frequently in international assessments to characterise risks to human health. The risk characterisation is conducted by comparing quantitative information on exposure to the NOAEL/NOAEC and deriving a MOE as follows:

1. Identification of critical effect(s).
2. Identification of the most appropriate/reliable NOAEL (if available) for the critical effect(s).
3. Where appropriate, comparison of the estimated or measured human dose or exposure (EHD) to provide a MOE:

   MOE = NOAEL/EHD.
4. Characterisation of risk, by evaluating whether the MOE indicates a concern for the human population under consideration.

The MOE methodology was used for characterising occupational and public health risk following exposure to HBCD.

The MOE provides a measure of the likelihood that a particular adverse health effect will occur under the conditions of exposure. As the MOE increases, the risk of potential adverse effects decreases. In deciding whether the MOE is of sufficient magnitude, expert judgment is required. Such judgments are usually made on a case-by-case basis and should take into account uncertainties arising in the risk assessment process such as the completeness and quality of the database, the nature and severity of effect(s) and intraspecies and interspecies variability.

A MOE of 100 or greater is usually not considered a flag for concern as it represents the conservative default uncertainty factors of 10 each for both intraspecies and interspecies variability used for risk characterisation.

10.1 Critical health effects

HBCD is of low acute toxicity via the oral, dermal and inhalation routes. It is not an eye or skin irritant in rabbits or a skin sensitiser in animals or humans.

For repeat dose toxicity, there is no human data available to identify a robust NOAEL or profile the systemic toxicity of HBCD. Animal data are not available for the dermal and inhalation routes of exposure. In oral repeat dose toxicity studies in rats, effects of HBCD were observed on liver, thyroid, pituitary, immune system and bone density. The most common effect observed was increase in liver weights, which was mostly dose-dependent but reversible, except at high doses. In one 28 d dietary study, increased liver weight was accompanied by induction of some liver enzymes in female rats, leading to the conclusion that effects on liver and thyroid could be due to enzyme induction. A BMDL of 22.9 mg/kg bw/d for liver weight using 20% weight increase as the critical effect level was obtained in this study (Van der Ven, 2006). Data from this study (Table 8.4) indicated that liver weight increases in female rats were apparent from 30 mg/kg bw/d onwards, giving a low observed adverse effect level (LOAEL) of 30 mg/kg bw/d and a NOAEL of 10 mg/kg bw/d.

HBCD did not cause in-vivo genotoxicity or carcinogenicity. In the only chronic study, dietary exposure to mice over 18 months did not result in increased incidences of any tumors.

HBCD showed effects on some fertility and developmental parameters. Decreased size of thyroid follicles were also noted in these animals. In addition, a dose-dependent pup mortality during lactation was reported in the F2 generation animals. A NOAEL of 10.2 mg/kg bw/d was established in the 2-generation reproductive toxicity study in rats based on pup mortality during lactation in the F2 generation and a significant increased incidence of animals with decreased size of thyroid follicles in the 2 highest-dose groups in both sexes. While a dose-dependent decrease in the number of primordial follicles in the mid- and high-dose groups was observed in rats, this was not considered to be an adverse effect, as the number always remained within the historical control range.

There were indications of developmental neurotoxicity (altered spontaneous behaviour) in a study in which 10-day-old mice were exposed to low doses of HBCD (Eriksson et al., 2006). However, this study is not considered for risk characterisation, as it was not performed according to OECD guidelines. Risk for this effect will need to be assessed if futher studies confirm the developmental neurological effects of HBCD. Table 10.1 summarises the critical studies for determination of NOAEL for risk characterisation.

Table 10.1. Critical studies for determination of NOAEL for risk characterisation

Toxicity observed	NOAEL mg/kg bw/d	LOAEL mg/kg bw/d	Effect at LOAEL	Species and age at treatment	Reference
Repeat dose toxicity (28 d)	10.0	30 (BMD-L 22.9 mg/kg bw/d)	↑ liver, thyroid and pituitary weights, induction of hepatic CYP 2B	Adult rats	Van der Ven et al., 2006
Reproductive toxicity	10.2	101	↓ size of thyroid follicles in both sexes	Rats	Ema et al., 2008
	10.2	101	↑ Pup	Rats	Ema et al.,

			mortality during lactation		2008
Developmental toxicity rats	8.1–21.3	81–213	Hypothyroidism until adult stage. No abnormality in offspring	Pregnant rats (until day 20 after delivery)	Saegusa et al., 2009

Selection of NOAEL for risk characterisation

Based on the potential for long-term exposure of the general population to HBCD, a NOAEL from a long-term exposure study would be most appropriate for characterising risk to the general population. The NOAEL of 10.2 mg/kg bw/d from the 2-generation reproduction study was therefore used to determine the MOE.

It can be argued that selecting a NOAEL for risk characterisation from developmental studies would estimate risk only for a small section of the population (females of child-bearing age) and thus a NOAEL based on adverse effects observed in both male and female animals should also be used for estimating risk to the general population. The other pronounced effect of HBCD in animal studies was increase in liver weight in both male and female animals. The NOAEL for this effect in a reliable and well-controlled 28 d oral study was 10 mg/kg bw/d, which is very similar to the NOAEL from the reproductive study. Therefore, the risk calculated using this NOAEL would also cover risk from repeated exposure in the occupational use situation as well as to the general population, especially since HBCD is known to be persistent.

MOE calculations

The MOE for each exposure scenario was determined for inhalation exposure using measured exposure data and exposure values derived from EASE model and the NOAEL of 10.2 mg/kg bw/d established in a 2-generation reproductive toxicity study in rats.

A NOAEL for exposure via the inhalation route was not available to determine the inhalation risk to workers conducting repackaging and compounding. However, since HBCD administered orally was determined to be completely absorbed (100% absorption), the NOAEL from an oral study was used to estimate risk from inhalation of HBCD during occupational handling. For determining risk from dermal exposure, modelled dermal exposure data were converted to internal dose using the dermal absorption values for powder, granules and liquid formulations. Total exposure was determined to be the sum of the internal dose determined from dermal exposure values and dermal absorption rate and inhalation exposure, assuming 100% absorption from respiratory tract.

10.2 Public health risk estimates

Consumers using articles comprised of textiles or plastics treated with HBCD may be directly exposed to HBCD that is released from the articles. The potential risk of adverse effects following exposure to HBCD has been considered separately for adults and children since the routes of exposure may differ for each group.

HBCD has a low vapour pressure and significant emission from treated articles is not expected. Dermal exposure is possible through contact with HBCD-treated textile; however, an estimation of dermal exposure from automotive upholstery indicated very low dermal exposures for adults as well as for young children.

The highest calculated exposure to HBCD is via food, particularly for infants and toddlers. However, the calculation of exposure via food used a methodology that was believed to greatly overestimate exposure, because the highest HBCD concentration found in any food was then attributed to all foods. In spite of this methodology, all MOEs were found to be above 3000, indicating a low risk from exposure through food.

Exposure to HBCD through inhalation and ingestion of indoor dust was generally the next highest contributor to exposure. Properties of the indoor environment have a large influence on intake via this route, with nearly a 20-fold difference between the reasonable worst-case estimate of exposure of the general public and the estimate for a maximally exposed individual living in a house with one of the highest measured HBCD concentrations in dust.

Table 10.2 provides a summary of the exposure data and MOEs when the overall daily exposure is compared with the NOAEL of 10.2 mg/kg bw/d. As shown in the table, the MOEs for all population groups are greater than 3000, even without further refinement of the food intake methodology, indicating very low risk.

Table 10.2. Calculated margin of exposure (MOE) from combined direct and indirect exposure to HBCD

	HBCD Exposure (ng/kg bw/d)								MOE[1]
	Direct exposure			Indirect exposure				Total	
	Oral	Inhalation	Dermal	Total indoor exposure[2]	Total outdoor exposure[2]	Food consumption	Breast milk		
Infants	Negl.	Negl.	0.04 (w-c)	0.04 (typ) 0.28 (w-c)	0.001	Negl.	38 (typ) 124 (w-c)	38 (typ) 124 (w-c)	>10 000 >10 000
Toddlers	Negl.	Negl.	0.04 (w-c)	35 (typ) 231 (w-c)	4.8	24 (typ) 50 (w-c)	Not estimated	59 (typ) 286 (w-c)	>10 000 >10 000
Children	Negl.	Negl.	0.03 (w-c)	4.9 (typ) 32 (w-c)	0.7	6 (typ) 12 (w-c)	Not estimated	11 (typ) 45 (w-c)	>10 000 >10 000
Adults	Negl.	Negl.	0.03 (w-c)	20 (typ) 13 (w-c)	0.3	5.9 (typ) 12 (w-c)	Not estimated	8 (typ) 25 (w-c)	>10 000 >10 000

[1]MOE = NOAEL/total exposure. MOEs calculated based on a NOAEL of 10.2 mg/kg bw/d from a 2-generation reproductive study.
[2]Inhalation and ingestion of dust/soil.
Negl. = negligible.
typ = typical.
w-c = reasonable worst-case.

10.3 Occupational health risk estimates

HBCD is not manufactured in Australia but imported in powder or granular forms or as an aqueous dispersion. A major amount is also imported in EPS and XPS.

Workplace activities related to HBCD are repacking, formulation, use in textile treatment, polystyrene manufacture and use of end products. Formulation of products containing HBCD is reported to be an enclosed, automated process with closed mixing tanks employed. Similarly, textile treatment is essentially reported to be an enclosed and automated or semi-automated process. Liquid dispersions used for treating textiles contain HBCD at less than 2% concentration.

Potential routes of exposure to HBCD in the occupational setting are via inhalation and dermal contact. The likelihood of exposure by ingestion in occupational settings is likely to be low. The very low vapour pressure of HBCD means that the main routes of exposure are likely to be dermal and inhalation of dust. There is little potential for exposure where HBCD is used encapsulated in a plastic matrix.

Although powder formulations are currently not used in Australia, risks from powder formulations were estimated because these formulations could be imported and used in future, if not regulated.

10.3.1 Risk from physicochemical hazards

HBCD is a non-flammable solid (powder or granules) that does not undergo autoignition and has no evidence of explosive properties. It has a melting point of 175 ^{o}C to 190 ^{o}C and decomposition occurs at >190 ^{o}C.

HBCD is stable under normal storage conditions. Based on the properties of HBCD the risk from physicochemical hazards during storage and handling of HBCD is considered to be low.

10.3.2 Acute risks due to occupational exposure

HBCD has low acute oral, dermal and inhalation toxicity. The LD_{50} is greater than 20 g/kg for both dermal and oral routes of administration, and LC_{50} greater than 200 mg/L by the inhalation route. The toxicological profile of HBCD indicates that it is not a skin or eye irritant or a skin sensitiser. The risk of acute effects such as inhalation toxicity, skin, eye and respiratory irritation when handling technical HBCD or products containing HBCD is considered to be low.

10.3.3 Chronic risks due to occupational exposure

Model for exposure estimates

Measured and modelled data were used to estimate inhalation and dermal exposure for workers engaged in repackaging, mixing and compounding tasks. Overseas monitoring data were used to determine risk to workers during the repackaging, weighing and addition tasks. Short-term and full shift measurements were available for the addition task, and full shift measurement for the weighing task. The 50th and 90th percentiles were used as the typical and worst-case scenarios to estimate the risk during these tasks.

No measured exposure data were available for workers formulating liquid coatings and treating fabric using the liquid formulation. The dermal exposure for workers conducting these tasks was determined using the EASE model. The internal dose was estimated using the EASE results, dermal absorption rates and concentration of HBCD in the formulations. Dermal exposure for upholstery workers was also estimated using the EASE model.

Inhalation, dermal and total exposure and MOE for workers conducting the various tasks in typical and worst-case scenarios are presented in Table 10.3.

Table 10.3. Inhalation, dermal and total exposure and MOE for workers conducting the various tasks

Industry	Task	Internal inhalation exposure (μg/kg bw/d) (measured)		Internal dermal exposure (μg/kg bw/d) (modelled)		Total internal exposure (μg/kg bw/d)		MOE[1]	
		Typical	Worst-case	Typical	Worst-case	Typical	Worst-case	Typical	Worst-case
Importation and repackaging	Repackaging (powder)	161	216.4	57	570	218	786	46.8	13.0
	Repackaging (granules)	2.96	13.76	29	290	2.96	304	319	33.6
Polymer industry	Addition (powder)	2.5	10.2	57	570	59.5	580	168	17
	Weighing (powder)	58	98	57	570	115	668	87	15
	Addition (granules)	0.74	3.44	29	290	29.7	293	337	34
	Weighing (granules)	0.74	3.44	29	290	29.7	293	337	34
Textile industry	Formulation	None	None	34	340	34	340	300	30
End uses	Upholstery	None	0.75	Negligible	Negligible	Negligible	0.75	Not	13 600

	(modelled)	estimated
Building	Negligible (modelled)	

[1]MOE = NOAEL/Total Internal Exposure. MOEs calculated based on a NOAEL of 10.2 mg/kg bw/d from a 2-generation reproductive toxicity study.

10.3.4 Risk estimates for specific occupations

1) Importation, transport and repackaging

The risk of harmful effects by inhalation to workers handling the powder or granular forms of HBCD during importation and transport is likely to be negligible except in the case of breached packaging or accidental spillage. Based on the exposure estimates determined from measured data for inhalation and modelled data for dermal contact, the risk to workers repackaging HBCD powder in both typical and realistic worst-case scenarios and the granular form for realistic worst-case scenarios was found to be unacceptable.

2) Polymer industry

MOEs during weighing of HBCD powder or granules and adding to the reactor (compounding) were determined using measured data for inhalation and modelled data for dermal contact. Based on the exposure data, exposure during weighing HBCD powder could be high, resulting in a risk of adverse effects during this process. The MOEs during weighing of the granular form and compounding powdered and granular forms were higher than 100 for the typical scenarios, indicating low risk to workers. However, for the worst-case scenarios for all of these processes, MOEs were considerably lower than 100, indicating high risk to workers using powdered as well as granular HBCD.

MOE from measured data

In a study measuring the occupational exposure to powdered HBCD among workers at an industrial plant producing EPS, Thomsen et al. (2007) reported a mean level of 101 ng/g lipids HBCD in workers following a 8 h shift (see Section 6.5.1). In order to calculate the risk to these workers, the authors first converted the $BMDL_{10}$ of 22.9 mg/kg bw/d (obtained in the Van der Ven et al. study, 2006) into "ng/g liver lipids". This gave a value of 192 000 ng/g liver lipids. When this value was compared with the mean HBCD levels measured in workers' serum (101 ng/g lipids), a MOE of 1900 was calculated.

The difference in the MOEs obtained from the measured data in this study and the estimated data could be due to a number of reasons. Workers in this study were reported to be wearing appropriate PPE, which would effectively reduce exposure to HBCD. In addition, HBCD-containing products were produced at intermittent periods in the factory where the study was conducted (only 8 to 10 times a year), and the production period lasted only up to 14 d. Modelled data does not take PPE into account. Also, the study used a $BMDL_{10}$ of 22.9 mg/kg bw/d, whereas the present assessment has selected a lower NOAEL of 10.2 mg/kg bw/d based on reproductive toxicity of HBCD.

3) Textile industry

In the textile industry, exposure of workers to HBCD could occur during formulation of flame-retardant coatings and exhaustion treatment of polyester and blinds material with the formulations. Risk from inhalation is unlikely to be significant, as HBCD is dispersed in water and no dust is generated.

The use of HBCD to coat blinds is a fully automated process; however, the exhaustion treatment of polyester is a semi-automated process and dermal contact with HBCD is possible. Data for inhalation exposure during textile treatment is not available. The risk from dermal exposure was estimated using the internal dose calculated from modelled dermal exposure data and a dermal absorption rate of 4% for HBCD. Based on the internal dose calculated for a worst-case exposure during formulation, the MOE was determined to be 30, indicating unacceptable risk to workers carrying out this task.

End-use products

The risk to workers handling end-use products containing HBCD is expected to be low, as these products contain HBCD at very low concentrations. Moreover, the HBCD is either incorporated into a plastic matrix or fixed onto fibres. The commonly used HBCD products in Australia are the EPS and XPS foam products in the building industry and treated textiles in upholstered articles and blinds.

Upholstery workers

HBCD-treated textiles are used for the manufacture of upholstered articles. The potential risk to upholstery workers from inhalation exposure exists when HBCD is released during cutting and sewing the HBCD-treated fabrics. An inhalation exposure of 0.75 μg/kg bw/d was estimated for these workers. Dermal exposure to HBCD from the end-use products was not estimated and is expected to be very low. A MOE of 13 600 was calculated based on inhalation exposure.

Building industry workers

Building industry workers can be exposed to HBCD when installing EPS and XPS polystyrene boards. The installation of these boards involves cutting the boards to different sizes and shapes. Cutting of boards is accomplished either by hand-held instruments or with hot wire cutting implements. Risk from inhalation exposure is unlikely to be significant, as the board particles generated during cutting are not small enough to be inhalable and the concentration of HBCD in these boards is <2%. Similarly, risk from dermal exposure to HBCD leaching out from the treated material is expected to be very low.

10.4 Uncertainties in occupational risk assessment

Uncertainties in any risk characterisation process arise from data limitations, inadequate exposure information, assumptions made during the process and variability in experimental conditions. The uncertainties inherent in the characterisation of risk for HBCD arise mainly from inadequate data and include:

- absence of representative atmospheric monitoring in Australia
- absence of dermal exposure data
- lack of data on the health effects of HBCD in humans following repeated exposures
- use of a default oral NOAEL for determination of MOE estimates, as no reliable evidence of systemic toxicity was seen in dermal studies in a suitable animal model.

In addition, the assumptions used in EASE modelling add uncertainties to the risk characterisation.

10.5 Conclusions

HBCD is not manufactured in Australia. It is imported into Australia in various forms, such as the raw chemical, in liquid dispersions and as an ingredient of many products and articles.

The main occupational use of HBCD in Australia is in expanded and extruded polystyrene insulation panels. Other major uses are in the treatment of textiles for upholstery seatings, draperies and wall covering and in blinds. Minor uses include audio or video equipment housing and packaging material.

There is a lack of data on the use of consumer products containing HBCD in Australia. Data on the release of HBCD from consumer products or measured date of exposure of general public to HBCD present in articles or on treated textile is also lacking, and this does not allow a realistic exposure assessment. Consequently, exposure models have been used to determine the exposure that represents the greatest risk to consumers. Such models are not as reliable as measured data, as they invariably use conservative assumptions.

Overall, the available information indicates that, although public exposure will be widespread, it is at a very low level. The risk characterisation indicates that, under normal conditions of consumer use, the risk of adults and children being exposed to levels of HBCD that would lead to health effects noted in repeat-dose animal studies is very low. The risk characterisation indicates that the high potential sources of exposure include breast milk for infants and soil/dust for toddlers. Human breast milk data from the 1970s to 2000 show that HBCD levels have increased since HBCD was commercially introduced as a brominated flame retardant in the 1980s. However, international data indicate that the levels have since stabilised at levels posing minimal risks, and international action via the Stockholm Convention will further reduce ongoing risks.

Under occupational conditions, the risk to workers of acute adverse health effects such as inhalation toxicity, skin, eye and respiratory irritation and skin sensitisation is low.

In experimental animals, repeated exposure to HBCD causes adverse effects. Risk characterisation indicates that, for all tasks, the risk of adverse effects to workers is unacceptable for worst-case scenarios and there is need for risk reduction measures. In addition, the risk to workers repackaging powdered HBCD in a typical work scenario is significantly high (low MOE). Risk to workers handling semi-finished and end-use products is low, as these products contain HBCD at very low concentrations. Moreover, the risks are low, as HBCD is either incorporated into a plastic matrix or fixed onto fibres.

Generally, risk from dermal exposure is higher when compared to inhalation exposure, except when weighing and repackaging powder formulations where risks from inhalation is either similar or higher than that from dermal exposure.

Risk mitigating measures such as local exhaust ventilation and personal protective equipment are needed to protect workers from adverse effects. It has been estimated that cotton overalls and gloves worn during processes such as mixing/loading or repackaging reduce dermal exposure by up to 90% (Thongsinthusak et al., 1993).

11. Environmental risk characterisation

11.1 Introduction

The risk characterisation involves the calculation of a simple risk ratio (PEC/PNEC). Two outcomes are possible:

1) PEC/PNEC <1.

Where this is the result, the risk to the compartment under consideration is deemed acceptable and there is no need for further refinement of the PEC or PNEC, and no risk reduction measures are required. Where the PEC/PNEC is approaching 1, the margins of safety are reduced and increases in volumes of use may result in a PEC/PNEC exceeding 1.

2) PEC/PNEC >1

Where this is the result, the risk to the compartment under consideration is normally deemed unacceptable. However, there needs to be some flexibility in the interpretation of this ratio.

Two conclusions may be drawn from this outcome. It should be decided whether further information (including test data) would assist in helping to evaluate the risk (e.g. in the event the ratio is not significantly greater than 1) or, alternatively, if risk management measures are needed. In this case, the judgment should be made on the basis of the size of the PEC/PNEC ratio, and how risk management options may help to lower this ratio. HBCD has been judged to meet POPs persistence and bioaccumulation criteria, and it is very toxic to aquatic organisms. These factors must be taken into consideration when addressing risk management options.

11.2 Statement of uncertainties

There are large uncertainties surrounding the fate of HBCD in the environment and about the actual isomer composition found in the environment. The sources and interconversions in the environmental compartments remain to be clarified.

Release estimates have been performed for the current (limited) use patterns of HBCD in Australia. If use patterns alter or volumes imported in the future significantly increase then the current risk characterisation conclusions are unlikely to apply.

A cursory mass balance for HBCD shows landfill to be the major sink through disposal of articles at the end of their life (98% of imported HBCD predicted to be disposed of to landfill). Long-term releases of substances to the natural environment from controlled and uncontrolled landfill facilities, and the potential risk arising from this route, are issues unable to be addressed within the standard risk assessment methodology due to a lack of coordinated information across Australia.

11.3 Environmental risk estimates

11.3.1 Aquatic

A $PNEC_{aquatic}$ of 0.31 µg/L has been established for this risk assessment. Based on exposure concentrations determined, the risk quotients have been determined and are shown in Table 11.1.

Table 11.1. Aquatic risk quotients (PEC/PNEC) for different exposure scenarios

Situation	PEC_{river}	PEC/PNEC	PEC_{ocean}	PEC/PNEC
Plastics industry – processing (1 site only – technical grade granule)		NA[1]	0.06 µg/L	0.19
Automotive and technical textiles – processing (several sites, predominantly in Melbourne)		NA[1]	0.09 µg/L	0.29
Textile industry – processing, liquid dispersion, blinds (1 site only)		NA[1]	0.11 µg/L	0.35
Other local sources (plastics industry – EPS resin conversion; textile industry – automotive and technical textiles)		NA[1]	0.02 µg/L	0.06
End use (regional – background)	0.015 µg/L	0.05	0.0015 µg/L	0.005

[1] Not applicable for Australia, as these operations only occur at sites where release is to a coastal STP.

Using the risk quotient approach, no local unacceptable risk resulting from HBCD processing and end use has been identified.

11.3.2 Sediment

A $PNEC_{sediment}$ of 1 mg/kg dw has been determined for this risk assessment using the assessment factor approach. Based on default sediment characteristics, this equates to 0.38 mg/kg ww (noting the $PEC_{sediment}$ values are presented as wet weight levels), as shown in Table 11.2.

Table 11.2. Sediment risk quotients (PEC/PNEC) for different exposure scenarios

Situation	$PEC_{sediment}$	PEC/PNEC
Plastics industry – processing (1 site only – technical grade granule)	0.53 mg/kg	1.39
Automotive and technical textiles – processing (several sites, predominantly in Melbourne)	0.79 mg/kg	2.08
Textile industry – processing, liquid dispersion, blinds (1 site only)	0.97 mg/kg	2.55
Other local sources (plastics industry – EPS resin conversion; textile industry – automotive and technical textiles)	0.18 mg/kg	0.47

End use (regional – background)	0.013 mg/kg	0.03

Using the risk quotient approach, risk quotients exceeding 1 have been identified for local releases for the plastics industry – processing (1 site only, technical grade granule); from industry manufacturing end-use automotive and technical textile products containing HBCD; and for processing, liquid dispersions of HBCD into sun blinds (1 site only).

11.3.3 Terrestrial

A $PNEC_{soil}$ of 0.43 mg/kg dw has been derived. Using Level III fugacity model default values for soil (20% air, 30% water and 50% solids giving a wet weight density of 1500 kg/m^3), this corresponds to a $PNEC_{soil}$ of 0.34 mg/kg ww.

Exposure concentrations for the terrestrial compartment have only been derived for 2 scenarios, as shown in Table 11.3.

Table 11.3. Terrestrial risk quotients (PEC/PNEC) for different exposure scenarios

Situation	PEC_{soil}	PEC/PNEC
Soil amended with biosolids (1 year, local release)	0.45 mg/kg	1.32
Soil irrigated with effluent (10 years, local release)	0.072 mg/kg	0.21

No local unacceptable risk is indicated to the terrestrial compartment where soil is irrigated with effluent for up to 10 years. However, with the highest soil PEC (0.45 mg/kg soil, local operations for conversion of EPS resins in automotive and industrial textile operations with release to a single STP), the risk quotient exceeds 1, indicating a potential local risk to soil organisms.

The majority of raw HBCD (>95%) is used to produce flame-retarded EPS resins, and this is produced at only one site in Australia with release to one STP. Similarly, processing of liquid HBCD into blinds is only performed at a single site with release to one STP. While the number of sites processing liquid HBCD into automotive textiles is unclear, the volume is small and overall, the likely extent of any local soil risk through sludge amendment should be limited to sludge coming from a small number of STPs.

11.3.4 Atmosphere

In the air compartment, usually only an assessment on abiotic effects is possible and as such does not involve the determination of a PNEC, or therefore calculation of a risk quotient. If there are indications that one or more of these effects occur (e.g. long-range transport or global warming), expert knowledge from other areas (e.g. Australian Greenhouse Office and relevant international organisations) should be consulted.

HBCD has a modelled atmospheric half-life of 25 h (2.13 d). This indicates it has the potential to travel long distances in the air. In reality, the substance would mainly be sorbed to aerosol particles and, therefore, share the fate of these particles. However, it has been found in remote locations in both air and biota, therefore supporting a conclusion that it can undergo long-range transport.

11.4 Options to refine PEC and PNEC

At the screening level of assessment, there is a strong reliance on modelling to predict environmental exposures and, in many cases, environmental effects. Given the uncertainty arising from modelled results and compounding of this uncertainty when modelled results are used for further modelling, there are several possible test options available to refine the PEC and PNEC where the risk quotient shows an unacceptable risk.

Given the persistent and very bioaccumulative nature of HBCD, refinement of the PEC, even with further toxicity data, may not necessarily allow for more certainty in the risk assessment outcome in the wider sense.

11.4.1 Refinement of PEC

Further information may allow more refined PEC calculations. Such information could include:

1. A list of all sites undertaking end-use product manufacture using resins containing HBCD.
2. Monitoring information for Australia, particularly with respect to sediment levels.

A list of all manufacturing sites would allow better release estimations since STPs could then be identified and their characteristics taken into account in calculating local PECs. Such information would also allow refinement of the terrestrial PEC as better information on application of biosolids and effluent could be taken into account.

Monitoring information would enable a better understanding of local concentrations and hence, allow better refinement of the $PEC_{sediment}$. Such information would assist in refining the risk characterisation with respect to local release scenarios. However, it is unlikely to provide any further certainty for risk to the wider environment. A significant problem with HBCD is uncertainty over its environmental fate. There are studies that consider this aspect, but the results are difficult to interpret. There are uncertainties relating to the transformation of the isomers in the environment and difference in accumulation potential of each isomer.

11.4.2 Refinement of PNEC

The current suite of ecotoxicity data is limited for HBCD. This is particularly true for sediment organisms, and the $PNEC_{sediment}$ has been derived using equilibrium partitioning methodology and compared to the assessment factor approach where results are only available for one organism.

11.5 Conclusion

HBCD is toxic to both aquatic and terrestrial species with significant adverse effects on survival, reproduction and development reported in algae, daphnids and earthworms.

A risk quotient method that compares toxicity to environmental exposure was used to evaluate potential to cause harm to the environment. Modelling was used to determine predicted exposure concentrations in water, sediments and soil and PNEC for various media were derived using the assessment factor approach.

Using the risk quotient approach, the risk to aquatic species arising from use of HBCD in plastic or textile industry is low as indicated by risk quotients (RQ) of <1. However, the sediment risk quotients were >1 for most use scenarios of HBCD, indicating that HBCD concentrations in the Australian sediments have the potential to cause adverse effects. A potential local risk is also determined for terrestrial organisms from levels in soils amended with biosolids. However, this risk subsided to acceptable level for soils irrigated with effluent for up to 10 years.

Calculation of a risk quotient in air is not possible; however, it must be considered that the presence of HBCD in the atmosphere warrants concern in light of strong evidence that the substance is persistent and has the potential to travel long distances.

12. Current risk management

12.1 Human health risk management

This section discusses current regulatory controls and risk management practices in place to protect workers and the public from exposure to HBCD during manufacture and use of products containing HBCD.

12.1.1 Occupational health and safety

Control measures

According to the Safe Work Australia's *National model regulations for the control of workplace hazardous substances* (NOHSC, 1994a), exposure to hazardous substances should be prevented or, when this is not practicable, adequately controlled, so as to minimise risks to health and safety.

The Safe Work Australia's *National code of practice for the control of workplace hazardous substances* (NOHSC, 1994a) provides guidance in the form of a hierarchy of control strategies, namely:

elimination

substitution

isolation

engineering controls

safe work practices

personal protective equipment (PPE).

These measures are not mutually exclusive, and effective control usually requires a combination of these strategies.

As HBCD has been determined to be a hazardous substance in relation to human health according to the Safe Work Australia's *Approved criteria for classifcation of hazardous substances*, the following should be implemented to eliminate or minimise exposure to HBCD in the workplace.

Elimination and substitution

Elimination is the removal of a chemical from a process and should be the first option considered when minimising risks to health. In situations where it is not feasible or practical to eliminate the use of a chemical, substitution should be considered. Substitution includes replacing with a less hazardous substance or the same substance in a less hazardous form.

HBCD is not manufactured in Australia but is imported both as the raw chemical and as an ingredient in products. However, one applicant is reportedly considering evaluation of non-brominated fire retardants with the aim of replacing brominated chemicals currently used in textile treatment, including HBCD. Currently, no suitable replacement for HBCD in the EPS industry was reported to be available.

Isolation

Isolation as a control measure aims to separate employees as far as practicable from the chemical hazard. This can be achieved by the use of barriers or enclosures, or the use of automated processes. One applicant stated that, during the weighing process, HBCD powder or granules are manually poured through a hatch, and the blending process is fully enclosed. However, isolation of the repackaging process varies from open to semi-enclosed areas at various sites and a semi-automated process for compounding. These processes were therefore not completely isolated.

Engineering controls

Engineering controls are used in plants or processes to either minimise the generation and release of hazardous substances or limit the area of contamination in the event of spills or leaks. They include complete or partial enclosure, local exhaust ventilation (LEV) and automation of processes.

Data provided by one company treating textiles indicated that the treatment process was fully automated and carried out in well-ventilated open spaces. LEV was installed in the mixing room and around mixers where HBCD was added to the treatment product. The weighing rooms, where HBCD powder or granules were weighed, were equipped with dust extraction units. Dust extraction units were also used during the manufacture of EPS resin, and in the polypropylene and polystyrene masterbatch compounding processes in which powdered or granular HBCD was used. A hood extraction system with a shute was used by workers when manually handling the raw material in the manufacture of EPS resin. The production of plastic articles by injection moulding was usually a closed or semi-closed process and therefore no significant exposure to HBCD was expected.

These engineering controls appear to be adequate in protecting workers from exposure to HBCD.

Safe work practices

Safe work practices are administrative practices that enable people to work in a safe environment. These include avoiding prolonged skin contact, minimising inhalation of dust and removal of contaminated clothing. The use of PPE during repackaging, formulation and application of the product and compliance with label and MSDS information contribute to a safe working environment. Some of the current safe work practices are presented below:

- In the absence of LEV the various work processes were conducted in a well-ventilated area.
- Safety and emergency procedures were detailed in the relevant MSDS.
- MSDS were easily accessible to workers.
- Written procedures for use of the mechanical equipment were provided.
- Spillages were cleaned up promptly.

Disposal of waste was reported to be according to State government or accepted guidelines.

Personal protective equipment

PPE is used to minimise exposure to chemicals during packaging, mixing and/or formulation/manufacture.

From information submitted, some companies reported that workers handling HBCD raw material during the manufacture of EPS were supplied with long-sleeve shirts, trousers, overalls and safety shoes or boots. Additional PPE worn at that site included helmet, safety glasses and leather or chemical resistant gloves. Disposable dust masks and half- and full-face canister respirators were also available to operators if they wished to use them. Some appplicants advised their workers to use approved air purifying respirators if respiratory protection was required for certain operations. High airborne concentrations require the use of air supplied or self-contained breathing apparatus. Gloves and dust masks were used in the repackaging process.

Hazard communication

Labels

The *National code of practice for the labelling of workplace substances* (NOHSC, 1994b) is applicable to labels for workplace substances. Labels of consumer products are required to comply with the *Standard for the uniform scheduling of medicines and poisons* (SUSMP) (Australian Government, 2010). Labelling is a well-accepted and effective method for the provision of workplace information.

HBCD is currently not classified as a hazardous substance and is not listed in the Safe Work Australia's Hazardous Substances Information System (HSIS).

Material safety data sheets

MSDSs are the primary source of information for workers handling chemical substances. Under Safe Work Australia's *National model regulations for the control of workplace hazardous substances* (NOHSC, 1994a) and the corresponding State and Territory legislation, suppliers are obliged to provide an MSDS to their customers for all hazardous substances, as an MSDS is a well-accepted and effective method for the provision of workplace information.

Employers must ensure that an MSDS for any hazardous substance used in the workplace is prepared in accordance with the NOHSC *National code of practice for the preparation of material safety data sheets* (NOHSC, 2003) and readily accessible to employees with potential for exposure to the substance. A sample MSDS for HBCD prepared in accordance with this Code is provided in Appendix 2. This sample MSDS is for guidance only. Under the Safe Work Australia MSDS Code, manufacturers and importers have the responsibility of compiling their own MSDSs and ensuring that information is up-to-date and accurate.

HCBD has been determined in this assessment to be a hazardous substance in accordance with Safe Work Australia's *Approved criteria for classifying hazardous substances*. The criteria for classifying substances in the Approved Criteria document are based on human health effects. The environmental assessment of HBCD has indicated that it is very toxic to aquatic life with long-lasting effects.

A number of MSDS for HBCD and HBCD-containing products were provided for assessment. MSDSs provided for assessment fall into 3 main categories:

1) HBCD raw chemical

2) HBCD contained in textile treatment products

3) HBCD contained in expandable styrene.

The content and format of MSDSs for HBCD, as technical grade or in products and mixtures, were assessed according to *National code of practice for the preparation of material safety data sheets, 2nd Edition* (NOHSC, 2003). This assessment focused on the adequacy of the information provided in relation to the "core" elements; product identification, health hazard information; precautions for use; and safe handling information. The quality and adequacy of information presented in MSDSs for HBCD is summarised below.

Assessment of MSDSs for HBCD

A total of 11 MSDSs were provided for assessment. All but 1 MSDS attempted to cover the majority of core elements, but there was inconsistency between the MSDSs in the information provided.

MSDS for Technical HBCD

One MSDS did not follow the 16-header format recommended in the Code of Practice and another did not supply the name and address, telephone, or emergency telephone number for the Australian supplier.

None of the MSDSs reported that HBCD was not listed in the Poisons Schedule (*Standard for the uniform scheduling of medicines and poisons* (SUSMP)).

MSDS for HBCD products and mixtures

Three MSDSs did not follow the 16-header format and 2 did not provide information on use of the products.

While all MSDSs disclosed the presence of HBCD by chemical or generic name, 3 failed to provide the concentration of HBCD in the mixture. Two MSDSs did not contain information such as Australian supplier name, address or telephone numbers.

Toxicological data provided in the MSDSs for pure HBCD concurred with the toxicological data of this assessment. However, it was noted that studies were not referenced.

12.1.2 Public health regulations

HBCD is not listed in the *Australian drinking water guidelines* (NHMRC, 2004) or in SUSMP (Australian Government, 2010).

12.2 Current environmental risk management

This section discusses current regulatory controls and risk management practices in place to protect the environment from exposure to HBCD during manufacture and use of products containing HBCD.

The management of environmental pollution and waste in Australia is regulated through individual State and Territory regulatory systems rather than at a national level and each State and Territory has legislative frameworks and strategies for managing emissions and environmental pollution to air, land and waters.

HBCD is not specifically regulated for transport under the National Road Transport Commission's Dangerous Goods Code (ADG Code) (NTC, 2007).

Disposal and waste treatment

Each Australian State and Territory provides statutory controls on waste generation and management. Environmentally hazardous chemicals classified as wastes are sent to licensed waste disposal contractors in accordance with State and Territory requirements. No specific waste disposal guidelines, standards or management issues were identified for HBCD wastes.

Alternatives to HBCD

HBCD is used as a flame retardant primarily in building insulation composed of expanded or extruded polystyrene foam and also in textiles and other products. Emissions from HBCD-containing materials will be a potential long-term source to the environment. A large volume of HBCD ends up in articles, mainly in polystyrene (XPS, EPS) used in the construction and building sector. Alternatives to use of HBCD include flame retardant substitution, resin/material substitution and product redesign (UN POPRC7 Report: Risk Management Evaluation on HBCD, October 2011).

Product redesign and alternative insulating material have been suggested for EPS and XPS, for which an alternative chemical to HBCD is not currently available. Product redesign can involve ensuring that insulation is not placed in contact with flammable material. The types of alternative insulating materials include polyisocyanurate and phenolic foams and insulating blankets (fiber batts or rolls) that may contain rock wool, fiber glass, cellulose or polyurethane foam. Technical alternatives to HBCD containing EPS/ XPS include: blown-in or spray-applied insulation materials such as rock wool, fiber glass, cellulose, or polyurethane foam. Loose-fill cellulose insulation is commonly manufactured from recycled newsprint, cardboard, or other forms of waste paper. Loose-fill insulation can also be poured in place by using materials such as vermiculite or perlite.

Technically and commercially feasible alternative chemicals to HBCD are available for textile treatment and high impact polystyrene (HIP) products. Several of these are halogen-free and are therefore considered to be better alternatives for the environment and health (ECHA 2009, SWEREA 2010, KLIF 2010).

Some of the chemical alternatives currently available are arylphosphates (for HIP) and ammoniumpolyphosphate and pentaerythritol for textile back coating. Brominated chemicals such as tetrabromocyclooctane and dibromoethyldibromocyclohexane, are also commercially available as flame retardants for use in EPS applications in North America. However there are concerns about the environmental or health properties of these substances, including persistence and bioaccumulative effects and endocrine and mutagenic effects in mammalian cells in vitro (LSCP 2006, BSEF 2011).

A new polymeric fire retardant has reportedly been developed recently (http://www.dow.com/licensing/newsletter/archive/2011/may/201104c.htm). The substance is claimed to be non PBT and suitable for processing in EPS and XPS. The company developing this product has reportedly started licensing this technology to FR manufacturers to produce commercial quantities,

The US EPA has launched a program called Partnership on Flame Retardant Alternatives to HBCD to evaluate chemicals that can substitute for HBCD. Through the voluntary partnership on HBCD alternatives, chemical manufacturers, associations representing building and construction materials manufacturers, state governments, nongovernmental organizations, and other interested parties will evaluate chemicals that might substitute for HBCD. The alternatives will be evaluated by factors such as cancer hazard potential, genotoxicity, persistence, and bioaccumulation, according to information presented at the meeting. A timeframe for the project has not been indicated.

APPENDIX 1 – Classification under the Globally Harmonized System of Classification and Labelling of Chemicals

In this report, HBCD has been classified against the *Approved criteria for classifying hazardous substances* (NOHSC, 2004) and, in the case of physicochemical hazards, the *Australian dangerous goods code* (ADG Code). However, classifications under the Globally Harmonized System of Classification and Labelling of Chemicals (GHS) will come into force when the GHS is adopted by the Australian Government and promulgated into Commonwealth legislation. GHS documentation is available at http://www.unece.org/trans/danger/publi/ghs/officialtext.html

1.1 GHS classification

Based on the data presented in this assessment, HBCD is toxic to mammals (developmental toxicity) and highly toxic to aquatic and terrestrial species, with significant adverse effects on survival, reproduction and development reported in algae (EC50 <0.1 mg/L), daphnids (21 d NOEC (growth) = 3.1 μg/L) and earthworms (EC10=21.6 mg/kg dw soil).

The classification of HBCD to the GHS is presented in Table A-1. As yet, the GHS for environmental toxicity only addresses acute and chronic aquatic toxicity.

1.2 Persistent Organic Pollutants assessment

The Stockholm Convention on Persistent Organic Pollutants (POPs) is a global treaty to protect human health and the environment. The convention contains criteria which address persistence, bioaccumulation potential, long-range transport and toxicity concerns. These criteria are used to identify substances that may be candidates for inclusion in the treaty. The Convention entered into force on 17 May 2004. Australia ratified the Convention on 20 May 2004, and obligations of the Convention entered into force for Australia on 18 August 2004. The Stockholm Convention requires parties under the Convention to take into account POPS characteristics when conducting assessments on new and existing chemicals.

Based on laboratory data and international environmental monitoring data, there is sufficient evidence to conclude that HBCD will persist in the environment and meets POPs criteria for persistence. Data provided through both laboratory testing and environmental sampling of biota show the chemical (particularly the α isomer) is highly bioaccumulative and can biomagnify through the food chain. HBCD meets POPs criteria for bioaccumulation.

HBCD is highly toxic to aquatic organisms and meets international criteria to class the substance as toxic. While numerical values for toxicity are not included in the POPs criteria, there is sufficient evidence from test data to indicate that adverse effects on environmental organisms can occur at low concentrations.

Summary

HBCD is considered to meet the criteria for it to be considered a candidate for listing as a potential POP chemical.

Table A-1. Classification of HBCD under the GHS

Health and environmental hazards	Classification	Hazard communication
Health hazards		
Toxic to reproduction	**Category 2**	**Symbol:**  **Signal word**: Warning **Hazard statement**: Suspected of damaging fertility or the unborn child. May cause harm to breastfed children.
Environmental hazard		
Acute toxicity	**Category 1**	**Symbol:**  **Signal word:** Warning **Hazard statement:** Very toxic to aquatic life.
Chronic toxicity	**Category 1**	**Symbol:**  **Signal word:** Warning **Hazard statement:** Very toxic to aquatic life with long-lasting effects.

Appendix 2 - Sample Material Safety Data Sheet for hexabromocyclododecane

Section 1 – Identification of the material and supplier

Product name

Hexabromocyclododecane

Other names

Cyclododecane, hexabromo

1,2,5,6,9,10-Hexabromocyclododecane

Hexabromocyclododecane

Recommended use

Flame retardant in plastic products, in polymer dispersions for flame-retarding textile products, in polystyrene thermoplastic resin, and in articles.

Company name

Address

State **Postcode**

Telephone number **Emergency telephone number**

Section 2 – Hazard identification

HBCD is classified as hazardous according to the Approved Criteria for Classifying Hazardous Substances

Risk phrases

R63 Possible risk of harm to the unborn child.

R64 May cause harm to breastfed babies.

Safety phrases:

- S22 Do not breathe dust.
- S60: This material and its container must be disposed of as hazardous waste.
- S61: Avoid release to the environment. Refer to special instructions / Safety Data Sheets.

Section 3 – Composition/information on ingredients

Chemical entity	Proportion	CAS number
Hexabromocyclododecane	≥96%–99.9% w/w	3194-55-6 or 25637-99-4

Section 4 – First aid measures

Swallowed: If swallowed do NOT induce vomiting. Wash out mouth with water. Drink plenty of water. Seek medical advice.

Eyes: If in eyes, hold eyelids apart and flush the eye continuously with clean running water for at least 15 minutes, or until advised to stop by the Poisons Information Centre or a doctor.

First aid facilities: Ensure eye bath and safety showers are available and ready for use.

For advice, contact a Poisons Information Centre (131 126) or a doctor at once.

Advice to doctor: Treat symptomatically and supportively, no specific antidote known.

Section 5 – Fire fighting measures

Suitable extinguishing media

Water spray, carbon dioxide or foam – no restrictions

Hazard from combustion products
Vapours of hydrogen bromide, bromine, carbon monoxide and carbon dioxide

Precautions for fire fighters and special protective equipment
Do not inhale explosion gases or combustion gases.
Use mouth and respiratory protective device.

Section 6 – Accidental release measures

Emergency procedures
Avoid eye or skin contact. Wear protective glasses. Use protective gloves to avoid skin contact. Keep unprotected persons away. Do not hose spills down drains, sewers or waterways. Contact local waste disposal company for disposal.

Methods and materials for containment and clean-up
Accidental leaks and spillages should be cleaned up promptly with absorbents and put into containers for disposal. Empty drums and packages and their residues should be disposed in accordance with government regulations.

Section 7 – Handling and storage

Precautions for safe handling
Wear suitable respiratory protective device when decanting larger quantities without extractor facilities.
After handling the substance, wash hands thoroughly. Avoid contact with skin and eyes. While using do not eat, drink or smoke.

Conditions for safe storage, including any incompatibilities
Store away from foodstuff, drinks and feeding stuffs. Store in a well-ventilated, cool, dry area. Keep containers tightly sealed.

Section 8 – Exposure controls / personal protection

National exposure standard: none for HBCD
No exposure standards allocated

10 mg/m^3 (for inspirable dust)

Engineering controls
Use in well-ventilated area. Use local exhaust ventilation at source of dust (e.g. when weighing out and blending HBCD powder and granular formulations).

Personal protective equipment
Eyes/face protection – wear safety glasses.
Skin protection – wear chemical resistant gloves.
Respiratory protections – wear dust mask when using powdered formulation.
The personal protective equipment used should be in accordance with Australian, Australian/New Zealand or other approved standards.

Section 9 – Physical description and properties

Appearance: White

Odour: None

Boiling point: Decomposes at >190 ºC.

Melting point: 190 ºC

Vapour pressure :6.27 x 10^{-5} Pa @21 ºC

Specific gravity: 2.38 g/cm^3

Flash point: Not available

Solubility in water: 0.0034 mg/L

Section 10 – Stability and reactivity

Chemical stability
Stable under normal conditions of handling and use. HBCD decomposes at >190 ºC.

Incompatible materials
None known.

Hazardous decomposition products
Vapours of hydrogen bromide, bromine and carbon oxides are likely to be released in a fire.

Hazardous reactions
No data.

Section 11 – Toxicological information

Acute effects:
Animal data indicate that HBCD has low acute toxicity by the oral, dermal and inhalation routes.
Eye: Not an eye irritant.
Skin: Not a skin irritant.
Sensitisation: Not a skin or respiratory sensitiser.

Chronic effects:
Following repeated exposure, systemic toxicity was observed in the liver, thyroid and prostate.

HBCD is not mutagenic and did not produce tumours in a poorly conducted study testing the carcinogenic potential of HBCD. It is classified as toxic to reproductive system and development. Possible risk of harm to the unborn child and to breastfed babies.

A study on neurotoxic effects in mice demonstrated some dose-related neurotoxic effects at low exposure levels; however, further studies are required to confirm these results.

Section 12 – Ecological information

Overall
Very toxic to aquatic life (Category: Acute I).
Very toxic to aquatic life with long lasting effects (Category: Chronic I).

Ecotoxicity

Based on standard toxicity testing, HBCD meets the criteria to class the substance as very toxic to aquatic life.

Persistence/degradability

Based on laboratory data and international environmental monitoring data, there is sufficient evidence to conclude that HBCD will persist in the environment.

Do NOT allow to enter water, wastewater or the soil.

Bioaccumulative potential

Available data indicate that HBCD is highly bioaccumulative and can biomagnify through the food chain.

Section 13 – Disposal considerations

Disposal methods and containers
Dispose of according to government regulations. Do not allow product to reach sewage system.
Empty containers must be disposed of as a chemical waste.

Special precautions for landfill or incineration
Contact local waste disposal authority for advice or pass to a licensed waste disposal company for disposal.

Section 14 – Transport information

UN Number
3077

UN proper shipping name
Environmentally hazardous substance, solid, not otherwise specified

Class and subsidiary risk
Class 9

Packing group
III

Special precautions for user
None

Hazchem code
Not applicable

Section 15 – Regulatory information

Listed on the Australian Inventory of Chemical Substances (AICS).

Section 16 – Other information

Date of preparation

Abbreviations/acronyms
NOHSC – National Occupational Health and Safety Commission
HSIS – Hazardous Substances Information System

Appendix 3 – Detailed calculations used in public exposure estimates

Scenario 1: Dermal exposure from automotive upholstery

The exposed surface area for adults is estimated by:

$$S_{derm} = 25\% \times \left[5690\,cm^2\,(trunk) + 1980\,cm^2\,(thighs)\right] = 1918\,cm^2 \quad (adults)$$

and

$$S_{derm} = 1918\,cm^2 \times \left(\frac{BW_{child}(kg)}{60\ kg}\right)^{3/4} \qquad (children\ groups)$$

Dermal exposure from treated automotive upholstery is estimated using the following equation:

$$U_{derm} = \frac{C_{derm} \times S_{derm} \times T_{derm} \times t}{BW \times 24} \times B_{derm}$$

The parameter used and the values derived from the equation are summarised in the following table:

Parameter	Description	Units	Infants (1–6 months)	Toddlers (2 years)	Children (12 years)	Adults
BW	Bodyweight	kg	5.8	12.9	46.9	60
B_{derm}	Dermal absorption rate	%	4%	4%	4%	4%
C_{derm}	Concentration of HBCD in the skin surface water layer	ng/cm^3	46.3	46.3	46.3	46.3
S_{derm}	Surface area of skin in contact with upholstery	cm^2	333	606	1594	1918
T_{derm}	Thickness of water film	cm	0.01	0.01	0.01	0.01
t	Duration of contact	hr	1	1	1	1
U_{derm}	**Dermal uptake**	**ng/kg bw/d**	**0.04**	**0.04**	**0.03**	**0.03**

Scenario 2: Inhalation exposure from indoor and outdoor air

The exposure arising from the inhalation of indoor or outdoor air can be derived by using the following equation:

$$U_{inh} = \sum \frac{C_{air} \times V_{inh} \times 0.75 \times t}{BW \times 24}$$

The parameters used and the values derived from the equation are summarised in the following table:

Parameter	Description	Units	Infants (1–6 months)	Toddlers (2 years)	Children (12 years)	Adults
BW	Bodyweight	kg	5.8	12.9	46.9	60
V_{inh}	Ventilation rate	m^3/day	0.8	3.8	15	22
Exposure to HBCD in indoor air						
C_{indoor}	Concentration of HBCD in indoor air (dust)	ng/m^3	0.49 (typ) 3.21 (w-c)	0.49 (typ) 3.21 (w-c)	0.49 (typ) 3.21 (w-c)	0.49 (typ) 3.21 (w-c)
t_{indoor}	Duration of exposure (indoors)	h/d	20	20	20	20
U_{indoor}	Inhalation uptake via indoor air	ng/kg bw/d	0.04 (typ) 0.28 (w-c)	0.09 (typ) 0.59 (w-c)	0.10 (typ) 0.64 (w-c)	0.11 (typ) 0.74 (w-c)
Exposure to HBCD in outdoor air						
$C_{outdoor}$	Concentration of HBCD in outdoor air	Ng/m^3	0.078	0.078	0.078	0.078
$t_{outdoor}$	Duration of exposure (outdoors)	h/d	4	4	4	4
$U_{outdoor}$	Inhalation uptake via outdoor air	ng/kg bw/d	0.001	0.003	0.003	0.004

typ = typical; w-c = reasonable worst-case.

Scenario 3: Oral exposure from ingestion of soil and dust particles

The oral exposure arising from the ingestion of soil and dust particles can be derived by using the following equation:

$$U_{oral} = \sum \frac{C \times R_{ing} \times t}{BW \times 24} \times B_{oral}$$

The parameters used and the values derived from the equation are summarised in the following table:

Parameter	Description	Units	Infants (1–6 months)	Toddlers (2 years)	Children (12 years)	Adults
B_{oral}	Bioavailability	%	100	100	100	100
BW	Bodyweight	kg	9.4	12.9	46.9	60
R_{ing}	Ingestion rate	mg/d	Negligible	100	50	25
Exposure to HBCD in indoor soil and dust						
C_{dust}	Concentration in dust	µg/g	5.45 (typ) 35.63 (w-c)	5.45 (typ) 35.63 (w-c)	5.45 (typ) 35.63 (w-c)	5.45 (typ) 35.63 (w-c)
t_{indoor}	Duration of exposure	h/d	20	20	20	20
U_{dust}	Uptake from oral exposure via dust	ng/kg/d	Negligible	35 (typ) 230 (w-c)	4.8 (typ) 32 (w-c)	1. 9 (typ) 12 (w-c)
Exposure to HBCD in outdoor soil and dust						
C_{soil}	Concentration in soil	µg/g	3.7	3.7	3.7	3.7
$t_{outdoor}$	Duration of exposure	h/d	4	4	4	4
U_{soil}	Uptake from oral exposure via soil	ng/kg/d	Negligible	4.8	0.7	0.3

typ = typical; w-c = reasonable worst-case

Scenario 4: Oral exposure from the consumption of breast milk

The oral exposures arising from the consumption of breast milk are derived by the following equation:

$$U_{milk} = \frac{C_{milk} \times \mathrm{FC} \times \mathrm{R}_{\mathrm{milk}} \times F \times t}{\mathrm{T}} \times B_{oral}$$

The parameters used and the values derived from the equation are summarised in the following table:

Parameter	Description	Units
U_{milk}	Uptake from ingestion of contaminated breast milk	Ng/kg bw/d
C_{milk}	Concentration of HBCD in breast milk	ng/g lipid wt
FC	Fat content or lipid content in breast milk	4%
R_{milk}	Ingest rate of breast milk for infants who are fully breastfed	g milk/kg bw/d
F	Exposure frequency	day/month
t	Exposure duration	Month
t_{ave}	Averaging time	Day
B_{oral}	Bioavailability of the ingested HBCD	%

Group	Bodyweight (kg)			Typical case		Reasonable worst-case	
	Girl	Boy	Mean	Milk intake (mL/d)	BW adjusted intake (mL/kg/d)	Milk intake (mL/d)	BW adjusted intake (mL/kg/d)
1 month	3.98	4.29	4.14	702	169.6	1007	243.2
3 months	5.40	5.98	5.69	759	133.4	1025	180.1
6 months	7.21	7.85	7.53	765	101.6	1059	140.6
Average			5.79		134.9		188.0

From the table above, the BW adjusted average intakes for the typical case and the reasonable worst-case are 135 and 188 mL/kg bw/d, respectively. If a factor of age is also taken into the calculation, the average intakes will be slightly lower. When combining the BW adjusted intake rates with a human milk density factor of 1.03 g/mL, the intake rate of breast milk for infants who are fully breastfed (R_{milk}) is calculated to be 139 g/kg bw/d and 194 g/kg bw/d for the typical and the reasonable worst-case, respectively.

	C_{milk}	FC	R_{milk}	F	t	t_{ave}	B_{oral}	U_{milk}
Typical case	6.9	4%	139	30	6	180	100	38
Reasonable worst-case	16	4%	194	30	6	180	100	124

Scenario 5: Intake from the biological monitoring measurements

The daily intake is derived by the following equation:

$$DI = \frac{0.693 \times m_{lipid} \times C_{plasma/serum}}{T_{1/2} \times B \times BW}$$

where

Parameter	Description	Units	Sample 1	Sample 2
$C_{plasma/serum}$	HBCD in biological sample	ng/g lipid wt	1.1	7.0
m_{lipid}	Mass of lipid	kg	16.1	16.1
$T_{1/2}$	Elimination half-life	day	64	64
B	Bioavailability via the exposure route	%	100	100
BW	Bodyweight	kg	60	60
DI	**Daily intake**	**ng/kg bw/d**	**3.2**	**20**

Appendix 4 – Experimental data considered for environmental assessment of HBCD

A4.1 Environmental fate

A4.1.1 Biodegradation

Aerobic

Ready biodegradation

HBCD was tested for ready biodegradation in a 28 d closed bottle test at a concentration of 7.7 mg/L by measuring dissolved oxygen uptake and expressing it as a percentage of the theoretical oxygen demand or chemical oxygen demand (Schaefer & Haberlein, 1996). The test article was a composite of 3 lots of commercial HBCD. This study was performed according to OECD TG 301D, OPPTS 3200, and GLP.

No biodegradation was observed; the percent biodegradation was 0% over the 28 d test period. HBCD is not ready biodegradable based on this test result.

Inherent biodegradation

As part of a larger study, the biodegradation of HBCD was investigated following a tiered experimental design (Davis et al., 2004). Initially, the transformation of ^{14}C-HBCD was determined under aerobic and anaerobic conditions using activated sludge taken from a municipal wastewater treatment plant. Subsequent studies addressed the transformation of ^{14}C-HBCD in soil and freshwater sediments and are discussed further below.

For all tests, non-radiolabelled HBCD was a composite sample from 3 manufacturers containing 8.68%, 6.12% and 85.19% of α-, β- and γ-HBCD, respectively. The composition of the radiolabelled HBCD was 7.74%, 7.84% and 81.5% of α-, β- and γ-HBCD respectively, with ^{14}C- HBCD labelled at the minimum at the 1, 5 and 9 positions. Stock solutions were made by dissolving the test substance in acetone.

Aerobic sludge

This part of the study was performed based on OECD Zahn-Wellens/EPMA test for assessing inherent biodegradability (OECD TG 302B). ^{14}C-HBCD was added to sealed glass vessel containing activated sludge and a defined mineral medium. Vessels were equipped with CO_2 traps to allow regular monitoring of $^{14}CO_2$ production. The nominal concentration of ^{14}C-HBCD added to the reaction mixtures was 3.6 mg/L. Reaction mixtures were incubated at ambient temperature (19–25 ^{o}C) for 56 d with continuous stirring. $^{14}CO_2$ was measured weekly. Concentrations of test substance and ^{14}C products in the reaction mixtures were determined on days 0, 7, 14, 28 and 56.

Concentrations of total HBCD decreased from 99.1% applied radioactivity (AR) to 77.7% AR after 56 d. The following results were found with respect to the individual isomers over the study period:

Table A4.1. Recoveries of HBCD and individual isomers

Day	0	7	14	28	56

α	8.8	8.4	8	7.9	8.2
β	8.8	7.7	7	7.1	6.4
γ	81.6	72.3	68.2	70.1	63.1
TOTAL HBCD	99.1	88.4	83.2	85.1	77.7

Formation of ^{14}C-products was negligible and remained <2% throughout the study. In the poisoned control, 60% degradation of HBCD was observed during the study period with a much greater level of ^{14}C-products found, reaching 24% AR after 28 d and 44% AR after 56 d. These products, however, were not identified.

No ^{14}C-volatiles were found in the headspace gases of the test sludge or the poisoned control activated sludge mixtures. The amount of $^{14}CO_2$ collected in the caustic traps reached around 2% and 5% after 56 d for the viable and control mixtures respectively. Recovery of radioactivity ranged from around 85% to 100% throughout the course of the study.

HBCD was shown to not be inhibitory to the digester sludge at the tested rate. HBCD cannot be considered inherently biodegradable based on this test.

Soil

The transformation of HBCD was determined in aerobic and anaerobic soils based on OECD TG 307 and GLP (Davis et al., 2003a). The test substance was a composite and contained 5.8% α-, 19.3% β- and 74.9% γ-HBCD. The studies were conducted in laboratory batch microcosms prepared with a surface soil (top 15 cm) from Northwood, North Dakota. The soil was classed as a sandy loam (USDA) with 64% sand, 20% silt and 16% clay. The pH was 6.4, CEC 19.2 meq/100 g, bulk density 1.11 g/cm^3 and moisture at 1/3 bar of 23.9%. The organic carbon was 1.8%.

Activated sludge was obtained from a wastewater treatment plant and concentrated by centrifugation. The solid content was determined to be 15%.

Soil microcosms were prepared and the moisture content was adjusted by weight to be within the range of 40% to 60% water holding capacity (WHC). The test vessels were sealed and acclimatised for 35 d at around 20 °C. The headspace of the viable microcosms was exchanged with ambient air weekly during this period.

HBCD was added to replicate test mixtures at a nominal level of approximately 25 µg/kg soil dw and mixed thoroughly into the soil.

Microcosms were incubated in the dark at around 20 °C for 120 d. Headspace gases in the viable aerobic microcosms were exchanged with laboratory air after 14, 80 and 101 d. Oxygen concentrations in the headspace of the viable microcosms were routinely measured to confirm aerobic conditions were maintained. Sampling of the soils was performed on days 0, 1, 7, 21, 48, 65 and 119 in the viable microcosms and days 0, 21, 65 and 119 in the abiotic controls. HBCD levels in soil were determined by HPLC/MS. At the conclusion of the study, bromide levels were measured in selected microcosms using ion chromatography, and the headspace of selected background (blank controls) and HBCD amended microcosms was analysed for volatile brominated products.

Generally, based on the oxygen levels, conditions could be considered aerobic throughout the test period. Oxygen concentrations in the headspace of the viable background microcosms remained at around 20% from day 14. In the viable test vessels, oxygen concentration was variable. On most sampling occasions in different replicates, the oxygen ranged from 14% to 22%. However, on one occasion, it was as low as 3% in one replicate (day 80) and 1% in one replicate (day 119).

Degradation of HBCD was observed throughout the test period in the viable test vessels (75% decrease over the test period) compared to much lower degradation (3% after 119 d) in the abiotic controls. The breakdown pattern observed is shown in the Table A4.2.

Table A4.2. Degradation profile of HBCD in viable and abiotic microcosms

	Viable		Abiotic	
Day	HBCD (ng/g)	% decrease	HBCD (ng/g)	% decrease
0	15.9	0	18.0	0
1	13.0	18	–	–
7	11.5	28	–	–
21	9.5	40	16.3	9
48	6.6	58	–	–
65	5.1	67	16.0	11
119	4.0	75	17.4	3

Based on the nominal concentration added to the test system (25 ng/g), 63.8% and 72% were found on day 0 in the viable and abiotic test systems respectively. Degradation kinetics appeared be first order. Plotting ln(concentration) against time results in a linear degradation pattern ($r^2 = 0.91$). Degradation rate constants were determined by subtracting the abiotic rate constant from the viable rate constant. A half-life of HBCD in the aerobic soil tested was calculated to be 63 d. While this study does appear to show degradation under the aerobic conditions tested, some issues need consideration. It is not possible to calculate a mass balance. In terms of nominal concentrations, the amount found on day 0 in the viable and abiotic microcosms was 63.6% and 72% respectively. From the data it is unclear whether the low recovery was due to a poor extraction method or other factors. However, there is a strong linear loss pattern in the viable system compared to the abiotic control, where levels remained consistent throughout the test. This is curious given the lower extent of degradation observed in other studies with aerobic sludge, and the lack of degradation observed in the following study using aerobic soil without amending with activated sludge, as in this study.

Data presented in the report indicate that the dominant γ-HBCD isomer did not convert to either the α or β isomers in this test system. However, it is unclear whether the test concentration was actually sufficiently high to enable accurate detection of these isomers. There were no detectable bromine fragment ion peaks at retention times other than the HBCD retention time – that is, no brominated degradation products were detected. The α- and β- isomers were not detected after day 0 in the viable microcosms. Unfortunately, no quantitation of bromide released from HBCD could be performed because the levels of bromide present in the background microcosms were 160- to 250-

fold higher than the theoretical concentration that could be released in the test vessels from the level of HBCD added.

As part of a larger experiment, the biodegradation of ^{14}C-HBCD was investigated in aerobic soil following OECD TG 307 (Davis et al., 2004). Non-radiolabelled HBCD was a composite sample from 3 manufacturers containing 8.68%, 6.12% and 85.19% of α- β- and γ-HBCD, respectively. The composition of the radiolabelled HBCD was 7.74%, 7.84% and 81.5% of α-, β- and γ-HBCD respectively with ^{14}C- HBCD labelled at the minimum at the 1, 5 and 9 positions. Stock solutions were made by dissolving the test substance in acetone.

A single surface soil was collected from the same site in North Dakota used in the previous study (Davis et al., 2003a) described above, and sieved (2 mm). A series of soil microcosms were prepared by adding nominal 50 g dw soil (58 g ww) to 250 mL serum bottles and adjusting the moisture content to around 20% by weight. This moisture content was around 50% WHC. The test bottles were sealed and the reaction mixtures acclimatized for 15 d at 20 ^{o}C. Following this period the headspace gases of the microcosms were exchanged with fresh air. ^{14}C-HBCD was added to replicate test mixtures at a nominal concentration of 3.04 mg/kg. Controls included a biologically inhibited control (heat sterilized), a benzoate control (mineralization assay) and a toxicity control (HBCD + benzoate). Microcosms were incubated in the dark at around 20^{o}C for 112 d. The oxygen concentrations were routinely monitored in the headspace of the soil reaction microcosms and adjusted if needed. Viable test mixtures were analysed on days 0, 7, 14, 28, 56, 86 and 112 while biologically inhibited controls were analysed on days 0, 14, 28, 56, 86 and 112. The soil mixtures were extracted overnight with acetonitrile. Following extraction, they were centrifuged and filtrates assayed by LSC and HPLC-RAM to measure ^{14}C-HBCD and ^{14}C-products. The benzoate and toxicity controls were analysed for $^{14}CO_2$ on days 8, 14, 22 and 30.

Oxygen measurements in the test microcosms were generally lower than those in the controls. In the abiotic control, oxygen concentrations in the headspace gas were around 20% at measurement times throughout the study. In the test microcosm, oxygen in the headspace gas was 20% at the start of the test. By day 65, it had fallen to around 11% and on day 76 and 90, oxygen was replenished. Prior to replenishment at day 90, oxygen concentration in the headspace gas was only around 5% and increased to 17% after addition of extra oxygen.

In the viable microcosms, the following degradation of HBCD was observed:

Table A4.3. Recoveries of HBCD and individual isomers (% AR) over the study period

Day	0	7	14	28	56	86	112
α	8.6	8.8	8.3	8.4	8.9	8.6	8.2
β	8.3	9.2	8.7	7.8	8.2	6.7	7.5
γ	80.7	81.0	78.8	79.2	79.5	71.9	72.6
TOTAL HBCD	97.6	99.0	95.8	95.4	96.6	87.2	88.2

Degradation was minimal over this time and almost all attributed to degradation of the γ-HBCD isomer. In the biologically inhibited control, negligible degradation was observed over the study period, with total HBCD being 109.7% AR at day 0 and 102.8% AR at day 112. The authors attribute the losses observed in the viable

microcosms as being due to non-degradative processes – that is, non-extractable adsorption, since the concentrations of ^{14}C products detected in the soil did not exceed 1% during the study. Concentrations of volatile ^{14}C-products in the headspace gas of selected microcosms were <1% AR, while concentrations of $^{14}CO_2$ remained <2% AR.

A comparison of mineralisation levels of benzoate in the benzoate control versus the toxicity controls showed the level of HBCD tested was not inhibitory to the soil microbial populations under the test conditions.

Based on this test of one soil type, HBCD is expected to be persistent in aerobic soils.

Water/sediment systems

Davis et al. (2003b) tested the transformation of HBCD in 2 aerobic water/sediment microcosms following OECD TG 308 and GLP. This study was later published (Davis et al., 2005). Laboratory batch microcosms were prepared with authentic water and sediment collected from 2 rivers in the eastern United States containing the following characteristics.

Table A4.4. Sediment/water characteristics for the 2 test systems

	Schuylkill River	**Neshaminy Creek**
Sediment		
Classification (USDA)	Sand	Sandy loam
% Sand	95	65
% Silt	4	20
% Clay	1	15
% OC	0.4	4.2
Microbial biomass (mg/kg)	111	547
Water		
pH	7.7	8
Conductivity (mmhos/cm)	0.39	0.64
Alkalinity (mg $CaCO_3$/L)	59	83
Total dissolved solids (ppm)	190	308

In addition to the viable microcosms, abiotic controls were prepared by steam sterilisation of the sediment/water mixture prior to addition of HBCD. The moisture content of the sediment in the Schuylkill River and Neshaminy Creek systems was 26% and 53%, respectively with a corresponding amount of 37 and 21 g dry weight sediment, respectively added to each microcosm. Mean water and sediment depths as measured at the end of the experiment were 4 and 1.5 cm, respectively, in both the viable and abiotic Schuylkill River microcosms. This compared with a mean water depth of 3.8 cm in both the viable and abiotic Neshaminy Creek microcosms and a mean sediment depth of 2.2 and 2.0 cm in the viable and abiotic Neshaminy Creek microcosms, respectively. The aerobic microcosms were pre-incubated in the dark at around 20 ^oC for 49 d and maintained by periodically exchanging the headspace of the microcosms with ambient air to replenish oxygen.

The test substance consisted of a composite of 3 commercial samples comprising 5.8%, 19.3% and 74.9% α-, β-, and γ-HBCD respectively. The stock solution was added near the centre of the sediment layer, several millimeters below the surface of the sediment at nominal concentrations 34 and 60 μg/kg (sediment dry weight) for the Schuylkill River and Neshaminy Creek systems respectively. The incubation following addition of the test substance period lasted 119 d.

At selected sampling times, microcosms were centrifuged to allow the water and sediment layers to be separated for analysis. Aqueous bromide levels were measured in selected microcosms at the conclusion of the study to determine if bromide was released from the HBCD added. Also, at the conclusion of the study, the headspace of selected blank and test microcosms was processed to collect possible volatile brominated degradation products.

According to Tebbutt (1992), aerobic reactions show oxidation–reduction potentials of >200 mV while anaerobic reactions occur below 50 mV. Redox measurements of the water layer in the Schuylkill River increased from around 344 mV prior to HBCD addition to around 423 mV at the end of the study. Therefore, in this system, aerobic conditions were maintained in the water throughout the study. In the Neshaminy Creek system, conditions seemed a little more reducing, with a redox potential in the water of around 70 mV prior to HBCD addition and 142 mV found at the end of the test. The pH of the water layer remained relatively stable throughout the study, ranging from 7.2 to 7.9 in the Schuylkill River system and 6.8 to 7.3 in the Neshaminy Creek system.

Little HBCD was found in the water column. In the Schuylkill River microcosms, 16%, 20% and 7% of the HBCD levels in the above table were attributable to water column concentrations at days 0, 1 and 7 respectively, with 14% of the day 0 concentrations for the abiotic system attributed to water column levels. It appears that no HBCD was present in the water column at any other sampling time (noting there was no sampling of the abiotic system at days 1 and 7). In the Neshaminy Creek microcosms, HBCD was found in the water column of the viable system at days 0 and 1 (22% and 4% of levels at these sampling times respectively) and at day 0 in the abiotic system at 18% of HBCD levels detected.

In the viable Schuylkill River microcosms, the α and β isomers were not detected after day 0, indicating that the major γ isomer did not convert to the α or β isomers in this test system (also an observation in the Neshaminy system). Also, no brominated degradation products were detected.

HBCD concentrations measured in the water and sediment layers of each microcosm were combined and reported on the basis of the dry sediment weight of the microcosm with the following results obtained.

Table A4.5. HBCD concentrations (ng/g dw sediment) and % losses over time

Schuylkill River Microcosm – Aerobic				
	Viable		Abiotic	
Day	HBCD (ng/g)	% decrease	HBCD (ng/g)	% decrease
0	31.9	0	31.3	0
1	11.2	65		
7	31.2	22		
21	3.2	90	29	7
47	1	97		
64	<0.5	>98	21.6	31
91	<0.5	>98		
119	3.3	90	20.7	34

Neshaminy Creek Microcosm – Aerobic				
	Viable		Abiotic	
Day	HBCD (ng/g)	% decrease	HBCD (ng/g)	% decrease
0	80.7	0	58.3	0
1	42.4	47		
7	19.1	76		
21	5.7	93	22	62
64	3	96	31.1	47
91	0.6	>99		
119	9.2	89	2.7	95

Half-lives for HBCD losses were calculated using the relationship $t_{1/2} = \ln(2)/k$. Loss of HBCD due to biological processes was determined by subtracting the rate of loss measured in abiotic controls from the rate of loss measured in viable reaction mixtures. This resulted in biodegradation half-lives of 11 and 32 d in the Schuylkill River and Neshaminy Creek systems respectively where the degradation kinetics were performed to days 64 and day 91 respectively. It is unclear (and no reasoning is offered by the study authors) why elevated levels of HBCD were found in the day 119 samples in both systems. However, if the day 119 levels are taken as outliers, possibly as a result of sample contamination, and removed from the regression equation, first-order degradation kinetics are apparent with r^2 values of 0.92 and 0.89 for the Schuylkill River and Neshaminy Creek systems respectively.

These half-lives compared to abiotic half-lives in the 2 systems of around 187 d (r^2 = 0.88) in the Schuylkill River system and around 31 d (r^2 = 0.80) in the Neshaminy Creek system. When in situ, the redox measurements of the Schuylkill River and Neshaminy Creek sediments were 151 mV (aerobic) and –18 mV (anaerobic) respectively. While sediment redox potential was not measured throughout the study, if the results reflected the in situ results, and given the chemical was added in the middle of the sediment layer, an anaerobic reduction mechanism cannot be ruled out. For example, Keum and Li (2005) investigated the possible application of zerovalent iron for the remediation of PBDEs. Six BDEs ranging from monoBDE to decaBDE were dissolved in ethyl acetate (1 mL, 50 mg/L) and applied on powdery iron or iron sulfide (5 g) in a vial. After 3 h and 0.5, 1, 2, 3, 5, 7, 14 and 40 d, BDEs were extracted and analysed both quantitatively and for structural confirmation. The results showed that a stepwise debromination was the dominant reaction in all congeners. The reaction rate constants of lower BDEs decreased as the number of bromines decreased. While it is difficult to directly relate these findings to HBCD, anaerobic reductive transformation from environmentally relevant reducing agents such as sulfide minerals (iron sulfide and sodium sulfide) that can be found in sediments may at least be a possibility.

HBCD loss was observed in both viable and abiotic sediments. In one system, the loss was appreciably faster (around 17 times) in the viable system than the abiotic system, with a similar degradation rate for both viable and abiotic microcosms in the other system. The results for the viable systems suggest that, in the environment, HBCD will not persist in viable anaerobic sediments.

While not discussed in the test report in any depth, an interfering peak was observed in the LC/MS chromatograms in the background microcosms for the Neshaminy Creek system, which corresponded to γ-HBCD. The concentration of this isomer in the aerobic microcosm was around 19.5 ng/g dw, or over half the nominal concentration added to the test microcosm. This could mean the sediment used for this test system was contaminated. Further, the elevated levels in both systems found at day 119 (compared to the 3 previous sampling times) are of concern, and it may be that the method of extraction was inadequate to properly enable a characterisation of degradation. The results from this study should be treated with some caution, as it is likely the degradation has been overestimated.

As part of a wider study, the biodegradation of ^{14}C-HBCD was investigated in aerobic and anaerobic freshwater sediments (Davis et al., 2004). Non-radiolabelled HBCD was a composite sample from 3 manufacturers containing 8.68%, 6.12% and 85.19% of α-, β- and γ-HBCD, respectively. The composition of the radiolabelled HBCD was 7.74%, 7.84% and 81.5% of α-, β- and γ-HBCD, respectively, with ^{14}C-HBCD labelled at the minimum at the 1, 5 and 9 positions.

The study design was based on OECD TG 308. Laboratory batch microcosms prepared with sediments and associated river water were dosed with ^{14}C-HBCD with tests performed for both aerobic and anaerobic sediments. The water and sediments were collected from the Schuylkill River (Valley Forge, Pennsylvania, USA). At the aerobic site, the sediment was classified as a sandy loam (USDA) and contained 59%, 28% and 13% sand, silt and clay, respectively, and 3.7% organic carbon (OC). Microbial biomass was 175.5 mg/kg and moisture was 32.0% at 1/3 bar. The overlying water had a pH of 7.6 with total dissolved solids of 126 ppm and conductivity of 0.36 mmhos/cm.

Aerobic sediments were collected from 0 to 3 cm depth and had a reported in situ redox potential of +187 mV. Sediments were sieved (2 mm) prior to testing.

Portions of sediment (28 to 30 g) were combined with 100 mL portions of the corresponding surface water, vigorously mixed then stored for equilibration periods 28 d (aerobic) and 33 d (anaerobic) in the dark at 20 °C. Following this period the headspace of the aerobic microcosms was exchanged with fresh air and the reaction mixtures prepared. The viable test mixtures contained nominal HBCD at 4.67 mg/kg sediment dw. In addition, biologically inhibited control mixtures (heat sterilised), benzoate control mixtures (to quantify microbial activity) and toxicity control mixtures (benzoate + HBCD to evaluate the impact of HBCD on microbial activity) were maintained. The microcosms were incubated at 20±2 °C in the dark. Oxygen in the headspace of the aerobic microcosms was routinely monitored at each sampling point. The oxygen concentration was maintained at approximately 12% by injection of oxygen when necessary. Sediment microcosms were analysed on days 0, 5, 12, 21, 28, 56, 84 and 112 for the aerobic microcosms and on days 0, 5, 7, 14, 21, 28, 56, 84 and 113 for the anaerobic microcosms. The viability and toxicity controls were analysed for $^{14}CO_2$ on days 8, 13 and 28 and on days 7 and 15 for the aerobic and anaerobic microcosms, respectively. The headspace gases of selected reaction mixtures were analysed for volatile ^{14}C-products. For analysis, the water and sediment layers of the microcosms were separated by centrifugation. Each phase was extracted overnight and centrifuged to separate solids from the solution, which was then filtered through nylon membrane filters, and the filtrate was analysed by LSC and HPLC-RAM.

The pH and redox potential of a subset of aerobic sediment microcosms were regularly measured throughout the study. During the test phase the pH increased from 6.7 to 7.4. The redox potential measured at the bottom of the water layer ranged from -1 to +62 mV. According to Tebbutt (1992), aerobic reactions show oxidation–reduction potentials of >200 mV, while anaerobic reactions occur below 50 mV. Based on this, it appears the study was not performed under fully aerobic conditions and, in fact, conditions were more indicative of anaerobic.

In the viable sediments, HBCD decreased from 95% to 53% AR after 112 d with the following results obtained for the individual isomers.

Table A4.6. Recoveries of HBCD and individual isomers (%AR) over the study period

Day	0	5	12	21	28	56	84	112
α	9	9.5	8.6	8.4	8.1	6.7	5	5.1
β	8	7.3	7.5	6.5	5.7	3.5	2.6	4
γ	78.4	73.4	70.4	56.3	57.3	39.6	42.8	44.1
TOTAL HBCD	95.4	90.2	86.6	71.2	71.1	49.8	50.4	53.3

During the 112 d incubation period, 3 ^{14}C-products with retention times indistinguishable to those observed in the sludge digester studies were detected in the viable sediment reaction mixtures (described further below). Product I (tetrabromocyclododecene, or TBCD) increased to 14% AR after 28 d before decreasing to around 5% by the conclusion of the study. Product II (dibromocyclododecadiene, or DBCD) reached a maximum of 32% after 56 d and remained fairly constant thereafter. Product III (1,5,9-cyclododecatriene, or CDT) was not detected until after 21 d and levels continued to increase through to day 112 (but still <10% AR by this time). $^{14}CO_2$ was <1% AR over the course of the study.

In the biologically inhibited sediment, limited transformation of HBCD occurred (around 15% degradation over 112 d). Over the first 28 d only Product I was detected at around 3% AR, rising to a maximum of 11% AR by day 112. By the end of the study, Product I and II accounted for 100% of the HBCD transformation observed in the biologically inhibited controls.

At the conclusion of the study total recovery of radioactivity was 98% for the viable and 101% for the inhibited sediments. Nearly all radioactivity remained in the sediment layer, with <2% AR typically measured in the aqueous layer.

The potential for HBCD to inhibit biological activity in the aquatic sediment was evaluated by examining the mineralisation of ^{14}C-benzoate. Mineralisation of this substance incubated aerobically and anaerobically without HBCD reached around 12% and 34% after 28 d and 15 d respectively. Mineralisation of benzoate was also noted in the sediments amended with around 4 mg/L HBCD (toxicity controls) with $^{14}CO_2$ ranging from 21% (day 28) to 37% (day 15), indicating no inhibition of the sediment by HBCD.

The degradation half-life in aerobic sediment was not determined in this study. However, plotting ln(concentration %AR) against time showed first-order degradation kinetics ($r^2 = 0.78$). A rate constant was determined to be 0.0059 and a half-life (through combined biotic and abiotic routes) determined to be 117 d. This value should be used with some caution:

1. The test was conducted for a period of time less than 1 half-life under these conditions.
2. Redox measurements at the bottom of the water layer during the test do not provide convincing evidence the conditions were aerobic. Thirteen measurements taken over the course of the study were all <63 mV, and most were <50 mV.

Nonetheless, this study is considered to be of good quality. Mass balance results showed good accountability for the test material, all 3 isomers were ably tested due to the higher test concentrations (that were shown to not be inhibitory to soil microorganisms at the test level) and metabolite formation was well described.

Anaerobic

Sludge

The biodegradation of HBCD was investigated following a tiered experimental design. Initially, the transformation of ^{14}C-HBCD was determined under aerobic and anaerobic conditions using activated sludge taken from a municipal wastewater treatment plant (Davis et al., 2004).

For all tests, non-radiolabelled HBCD was a composite sample from 3 manufacturers containing 8.68%, 6.12% and 85.19% of α-, β- and γ-HBCD, respectively. The composition of the radiolabelled HBCD was 7.74, 7.84 and 81.5% of α-, β- and γ-HBCD respectively, with ^{14}C- HBCD labelled at the minimum at the 1, 5 and 9 positions. Stock solutions were made by dissolving the test substance in acetone.

Results from the aerobic sludge component of this study (discussed above) showed very little degradation of HBCD over a 56 d exposure period. The anaerobic digester sludge part of the study is described here. This part was based on the ISO Standard 11734 test. ^{14}C-HBCD was added to anaerobic reaction mixtures containing digester sludge in a defined mineral medium. A portion of the collected digester sludge was diluted with the mineral medium to attain a target sludge concentration of 2130 mg/L suspended solids. The final pH of the inoculated medium was adjusted to pH 7.4. The nominal concentration of ^{14}C-HBCD added to the reaction mixtures was 4.2 mg/L and was added to duplicate test mixtures. The reaction mixtures were prepared in an anaerobic atmosphere and positive, negative and solvent controls maintained. The test was performed at around 35 ^{o}C in darkness. To determine degradation and metabolite production, samples were analysed on days 0, 5, 7, 14, 21, 28 and 60. Samples were extracted and assayed by LSC and HPLC-RAM. The headspace gases were monitored for volatile ^{14}C-products. Total gas production was measured on days 4, 10, 18, 28, 47 and 60. Methane concentrations were measured in selected microcosms on days 34 and 60 to confirm that methanogenic conditions existed in the digesters sludge mixtures. Total culturable organisms in the viable and biologically inhibited digester sludge reaction mixtures were determined by growth.

Low redox conditions were maintained (–100 mV) throughout the study. These values confirm anaerobicity (Tebbutt, 1992). The tested level of HBCD was shown to not be inhibitory to the digester sludge.

In the viable microcosm the concentrations of HBCD decreased from 114% AR to 15% AR by the conclusion of the study. The primary degradation for the 3 diastereomers ranged from 67% to 76% after 7 d and 87% to 92% after 60 d. The following results were found with respect to total HBCD and the individual isomers over the study period.

Table A4.7. Recoveries of HBCD and individual isomers (%AR) over the study period

Day	**0**	**5**	**7**	**14**	**21**	**28**	**60**
α	10.1	5	3.3	1.6	1.8	1.3	1.2
β	9.7	4.3	2.4	2	1.7	1.1	<1
γ	94.4	46.3	24.5	25.9	24.4	8.9	12.5
TOTAL HBCD	114.2	55.7	30.1	29.5	27.9	11.2	14.5

Further details of the study are presented in the full report.

Anaerobic sludge – supplemental studies

1. Davis et al., (2004) also performed a supplemental anaerobic digester sludge study to facilitate isolation and identification of transformation products. Reaction

mixtures were prepared with nominal HBCD concentrations of 0, 1, 50, 100 and 500 mg/L. ^{14}C-HBCD in acetone was mixed with HBCD in the bottles and the acetone was allowed to evaporate. Test vessels were incubated in the dark at 35 ^{o}C. Samples were removed on days 8, 14, 20, 35, 44, 69 and 93 and analysed for ^{14}C-HBCD and ^{14}C-products. After 106 d the remaining 0 and 500 mg HBCD/L mixtures were extracted overnight and degradation products identified.

The identification of these products was determined through a combination of HPLC/MS and GC/MS analyses. They were identified as tetrabromocyclododecene (Product I), dibromocyclododecadiene (Product II) and cyclododecatriene (Product III).

2. Hunziker et al. (2004) reported results of an anaerobic sludge study using HBCD. A composite of commercial HBCD (purity, 95%, 8.0%, 5.4% and 86.6% α, β and γ diastereomers respectively) was used along with a radioactive sample, ^{14}C-HBCD consisting of 7.7%, 7.8% and 81.5% α, β and γ diastereomers respectively. The anaerobic (digester sludge) studies followed the ISO Standard 11734 test. ^{14}C-HBCD was added at a nominal concentration of 4 mg/L, with a sludge concentration of 2000 mg/L. Anoxic reaction mixtures were prepared in an anaerobic atmosphere, sealed and incubated at 35 ^{o}C in the dark. Rapid degradation was noted under anaerobic conditions with the loss of approximately 50% of the ^{14}C-HBCD after 5 d. Degradation continued over the next 28 d, with approximately 90% transformation. Two major (unidentified) metabolites were found. The first reached a peak of 50% applied radioactivity after 7 d. By day 28 this product appeared to be declining and represented around 40% AR by day 28. The second major metabolite was found at around 10% AR after 7 d and remained at a similar level until day 28. Very little experimental details are provided with this report, and it is unclear whether they are actually preliminary results for the fuller test described by Davis et al. (2004) above.

3. Gerecke et al. (2006) reported the degradation of a technical HBCD mix and 6 HBCD stereoisomers under anaerobic conditions in sewage sludge. Heat sterilised samples served as negative controls. Incubation with a racemic mixture of α-hexachlorocyclohexane as a substrate served as positive controls. Experiments were conducted by adding individual compounds or mixtures to freshly collected digested sewage sludge from a mesophilic digester. In order to study different incubation conditions, nutrients and primers were added to some experimental set-ups. In addition, grab samples from the inlet and outlet of a full-scale anaerobic digester were analysed to verify the results from the laboratory experiments. For all racemic HBCD incubations, no primers were added. Each stereoisomer group ((±)-α-HBCD, (±)-β-HBCD and (±)-γ-HBCD)) was added at a concentration of 3.9 nmol (2.5 µg/L). The technical HBCD mixture was added at around 10 nmol (~6 µg/L). For all, nutrients of yeast (50 mg) and starch (20 mg) were added. In addition, the technical mixture had 4-bromobenzoic acid (around 10 nmol) added as a primer.

Actual degradation data were not provided in the report. However, for all test substances, degradation was fast. The technical HBCD mixture degraded with an apparent pseudo-first-order rate constant of 1.1 ±0.3/d, corresponding to a half-life of 0.66 d. This was not dependent on the presence of additional nutrients. There was a decrease in HBCD concentrations observed in the sterile control and a half-life of at least 35 d was found in this system. The (±)-β-HBCD and (±)-γ-HBCD degraded more rapidly than (±)-α-HBCD by an estimated factor of 1.6 and 1.8 respectively. A 7 h incubation sample from each of the set-ups was subjected to enantio-selective analysis and showed the enantiomeric fractions for α-, β- and γ-HBCD were essentially 0.5 (i.e.

all were racemic mixtures). There was no evidence that the degradation of HBCD was an enantioselective process in the test system used here.

Soils

The transformation of HBCD was determined in anaerobic soils based on OECD TG 307 and GLP (Davis et al., 2003a). This study was later published (Davis et al., 2005). The test substance was a composite and contained 5.8% α-, 19.3% β- and 74.9% γ-HBCD. The studies were conducted in laboratory batch microcosms prepared with a surface soil from Northwood, North Dakota. The top 15 cm soil was collected and sieved (2 mm). The soil was classed as a sandy loam (USDA) with 64% sand, 20% silt and 16% clay. The pH was 6.4, CEC 19.2 meq/100 g, bulk density 1.11 g/cm^3 and moisture at 1/3 bar of 23.9%. The organic carbon was 1.8%.

Activated sludge was obtained from a waste-water treatment plant and concentrated by centrifugation. The solids content was determined to be 15%.

Soil microcosms were prepared by adding 50 g dw soil to 250 mL serum bottles and transferred to an anaerobic glove box with an anaerobic atmosphere. 20 mL portions of steam sterilised tap water, which was previously sparged for 10 minutes with nitrogen, were added to each of the microcosms to cover the soil. They were then acclimatised for 43 d at around 23°C to allow methanogenic conditions to develop. Redox potential and pH were analysed in a subset of microcosms once per week and the headspace of selected microcosms was monitored for methane.

After acclimatisation, 1.7 g activated sludge was added to each microcosm. The final sludge concentration of 5 mg mixed liquor suspended solids per gram dry soil is representative of sludge application rates to soil for farming applications in Europe. HBCD was added to replicate test mixtures at a nominal level of approximately 25 µg/kg soil dw and mixed thoroughly into the soil. Biologically inhibited controls were prepared by steam sterilizing the soil on 3 separate days prior to the addition of the test compound. The activated sludge added to these controls received 1 steam sterilisation treatment.

Microcosms were incubated in the dark at around 20°C for 120 d. Headspace gases in the viable aerobic microcosms were exchanged with laboratory air after 14, 80 and 101 d. Sampling of the soils was performed on days 0, 1, 7, 21, 56, 91 and 119 in the viable microcosms and days 0, 21, 56 and 119 in the abiotic controls. At the sampling times, the microcosm soil was dried and extracted with hexane on a shaker for approximately 1 day. HBCD levels were determined by HPLC/MS. At the conclusion of the study, bromide levels were measured in selected microcosms using ion chromatography. The headspace of 5 microcosms (single viable background and duplicate test; single abiotic background and test) that were sealed for the entire study were sampled and analysed.

The pH of the water remained between 6.1-6.5 during the stabilisation phase and increased to 6.8 at the conclusion of the study. Redox potentials in the water remained in the range of 132-291 mV during the stabilisation phase. Resazurin dye, added as a visual indicator of redox potential, was colourless, indicating a low redox potential. The reason for the positive values measured with the redox probe is not known, however, they indicate more aerobic conditions (Tebbutt, 1992). The breakdown pattern observed is shown in the following table:

Table A4.8. Degradation profile in viable and abiotic microcosms

	Viable		Abiotic	
Day	HBCD (ng/g)	% decrease	HBCD (ng/g)	% decrease
0	11.0	0	17.2	0
1	8.0	27	-	-
7	8.5	23	-	-
21	0.9	92	19.1	0
56	0.5	95	11.8	31
91	nd	>95	-	-
119	nd	>95	6.9	60

Based on the nominal concentration added to the test system (25 ng/g), 44% and 68.8% were found on day 0 in the viable and abiotic test systems respectively. The reason for the low recovery in the viable test vessels is unclear and was not addressed by the study authors. In determining the degradation half-life, the authors used data points between days 0-21 (when 92% disappearance was observed). Plotting ln(concentration) against time to day 21 results in a linear degradation pattern ($r^2 = 0.92$). Degradation rate constants were determined by subtracting the abiotic rate constant from the viable rate constant. In the anaerobic study, over the 119 d, first order degradation was observed ($r^2 = 0.93$) and a rate constant of 0.0085 was determined giving an abiotic half-life of 120 d. The rate constant on the 0 d to 21 d data in the viable test vessels was 0.1153. Therefore, the rate constant for biotic degradation was 0.1153 - 0.0085 = 0.1068 resulting in a half-life of HBCD in the anaerobic soil tested of 6.49 d (6.9 d reported in test report, but probably due to rounding of the rate constant value).

It is noted that the level of HBCD found at day 56 (0.5 ng/g) was the LOD of analysis. If this level is maintained in the dataset, a linear degradation pattern is not so obvious, but still acceptable ($r^2 = 0.83$). In this case, the rate constant is 0.0563. Therefore, the half-life based on subtracting the rate constant found in the abiotic control is 0.0563 – 0.0085 = 0.0478, which results in a longer anaerobic half-life due to microbial degradation of 13.7 day.

HBCD degradation products were not detected in extracts of the soils. Unfortunately, no quantitation of bromide released from HBCD could be performed because the levels of bromide present in the background microcosms were 160-250 fold higher than the theoretical concentration that could be released in the test vessels from the level of HBCD added.

The results of this test should be treated with caution as there are questions over the actual aerobic/anaerobic nature of the test system. No reason is provided for the low recovery of nominal values. No mass balance is available, making it difficult to "follow" the chemical through the course of the study. Also, the very high levels of bromide present in the background microcosms did not allow quantitation of bromide released from HBCD, further weakening the results.

Water/sediment systems

Davis et al., (2003b) tested the transformation of HBCD in 2 anaerobic water/sediment microcosms following OECD TG 308 and GLP. This study was later published (Davis et al., 2005). Laboratory batch microcosms were prepared with authentic water and

sediment collected from 2 rivers in the eastern United States containing the following characteristics:

Table A4.9. Sediment/water characteristics

	Schuylkill River	Neshaminy Creek
Sediment		
Classification (USDA)	Loamy sand	Sandy loam
% Sand	87	74
% Silt	6	20
% Clay	7	6
% OC	2.5	4.2
Microbial biomass (mg/kg)	125	218
Water		
pH	6.5	6.7
Conductivity (mmhos/cm)	0.38	0.62
Alkalinity (mg CaCO3/L)	58	80
Total dissolved solids (ppm)	194	316

Anaerobic microcosms were prepared in an anaerobic atmosphere (70% N, 28% CO_2 and 2% H_2). The microcosms were pre-incubated at around 23°C for 43 to 44 d to allow the microcosms to stabilize.

In addition to the viable microcosms abiotic, controls were prepared by steam sterilization of the sediment/water mixture prior to addition of HBCD. The moisture content of the sediment in the Schuylkill River and Neshaminy Creek systems was 48 and 61% respectively with a corresponding amount of 20 and 14 g dry weight sediment respectively added to each microcosm. Mean water and sediment depths as measured at the end of the experiment were 3.9-4.04 cm and 1.6-1.7 cm respectively in both the viable and abiotic Schuylkill River microcosms. This compared with a mean water depth of 4.0 cm and a mean sediment depth of 1.7 cm in both the viable and abiotic Neshaminy Creek microcosms.

Regular measurements of pH and redox potentials in selected microcosms were used to confirm the stabilization of the systems. Additionally, the headspace of selected microcosms was monitored for the presence of methane during the stabilization phase.

The test substance consisted of a composite of 3 commercial samples comprised of 5.8%, 19.3% and 74.9% α-, β-, and γ-HBCD respectively. Acetone was used as a carrier solvent to introduce the HBCD into the test microcosms. The stock solution was added near the centre of the sediment layer, several millimeters below the surface of the sediment at nominal concentrations of 63 and 89 µg/kg (sediment dry weight) for the Schuylkill River and Neshaminy Creek systems, respectively. The incubation following addition of the test substance period lasted 119 d.

At selected sampling times, microcosms were centrifuged to allow the water and sediment layers to be separated for analyses. Aqueous bromide levels were measured in selected microcosms at the conclusion of the study to determine if bromide was released from the HBCD added. Also, at the conclusion of the study, the headspace of selected blank and test microcosms was processed to collect possible volatile brominated degradation products. HBCD concentrations were measured using HPLC/MS. Bromide concentrations were determined using ion chromatography.

In the viable Schuylkill River system, redox potential of the water layers immediately above the sediment layers showed a reduction from 151 mV at days 8 to 69 mV at day 44. According to Tebbutt (1992), aerobic reactions show oxidation-reduction potentials of >200 mV while anaerobic reactions occur below 50 mV. Therefore, these conditions cannot be described as truly anaerobic but are still probably reducing. By day 119, the redox potential was -75 mV in the viable microcosms, a level that is considered anaerobic. Similarly, in the viable Neshaminy Creek system, conditions were not truly anaerobic from day 7 to day 43 (90 mV and 66 mV respectively). However, by day 119, conditions were truly anaerobic with the redox potential measured at -424 mV.

HBCD concentrations measured in the water and sediment layers of each microcosm were combined and reported on the basis of the dry sediment weight of the microcosm with the following results obtained:

Table A4.10. HBCD Concentrations (ng/g dw sediment) and % losses over time

Schuylkill River Microcosm - Anaerobic				
	Viable		Abiotic	
Day	HBCD (ng/g)	% decrease	HBCD (ng/g)	% decrease
0	27.7	0	27.2	0
1	6.2	78		
7	nd	>98		
14	nd	>98	14.2	48
61	nd	>98	nd	>98
91	nd	>98		
119	nd	>98	nd	>98
Neshaminy Creek Microcosm - Anaerobic				
	Viable		Abiotic	
Day	HBCD (ng/g)	% decrease	HBCD (ng/g)	% decrease
0	39.1	0	37.5	0
1	55.9	0		
7	nd	>98		
14	nd	>98	14.2	62
62	nd	>98	nd	>98
91	nd	>98		
119	nd	>98	1.4	96

Little HBCD was found in the water column. In the Schuylkill River microcosms, 20% of the HBCD levels in the above table were attributable to water column concentrations at day 0. It appears that no HBCD was present in the water column at any other sampling time. In the Neshaminy Creek microcosms, HBCD was found in the water column of the viable system at days 0 and 1 (6% and 2% of levels at these sampling times respectively) and at day 0 in the abiotic system at 20% of HBCD levels detected.

The day 0 recoveries were only around 44% nominal in both systems. The reason for these low recovery systems is unclear. The authors suggest this indicated that a rapid removal mechanism was operative both biotically and abiotically. However, it could also be an indication of an inadequate method of extraction.

Half-lives for HBCD losses were calculated using the relationship $t_{1/2} = \ln(2)/k$. Loss of HBCD due to biological processes was determined by subtracting the rate of loss measured in abiotic controls from the rate of loss measured in viable reaction mixtures. This resulted in biodegradation half-lives of 1.5 and 1.1 d in the Schuylkill River and Neshaminy Creek systems, respectively.

These half-lives compared to abiotic half-lives in the 2 systems of around 10.4 d (r^2 = 0.99) in the Schuylkill River system and around 9.4 d (r^2 = 1) in the Neshaminy Creek system. While the redox potential of the sediments was not determined during the study, the *in situ* readings for both systems showed they were strongly anaerobic with redox measurements of around –200 mV for each.

HBCD loss was observed in both viable and abiotic sediments. In both systems, the loss rate in the viable system was considerably faster than in the sterile system, although HBCD did not persist in either. The results for the viable systems suggest that, in the environment, HBCD will not persist in viable anaerobic sediments.

While not discussed in the test report in any depth, an interfering peak was observed in the LC/MS chromatograms in the background microcosms for the Neshaminy Creek system, corresponding to γ-HBCD. The concentration of this isomer in the abiotic anaerobic microcosm was around 4.9 ng/g dw, or around 6% of the level added to the test microcosm. This could mean the sediment used for this test system was contaminated.

As part of a wider study, the biodegradation of ^{14}C-HBCD was investigated in anaerobic freshwater sediments (Davis et al., 2004). Non-radiolabelled HBCD was a composite sample from 3 manufacturers containing 8.68%, 6.12% and 85.19% of α-, β- and γ-HBCD, respectively. The composition of the radiolabelled HBCD was 7.74%, 7.84% and 81.5% of α-, β- and γ-HBCD respectively with ^{14}C-HBCD labelled at the minimum at the 1, 5 and 9 positions. Stock solutions were made by dissolving the test substance in acetone.

The study design was based on OECD TG 308. Laboratory batch microcosms prepared with sediments and associated river water were dosed with ^{14}C-HBCD. The water and sediments were collected from the Schuylkill River (Valley Forge, Pennsylvania, USA). At the anaerobic site, the sediment was classified as a sandy loam (USDA) and contained 61%, 28% and 11% sand, silt and clay respectively and 3.4% OC. Microbial biomass was 197.6 mg/kg and moisture was 24.9% at 1/3 bar. The overlying water had a pH of 6.6 with total dissolved solids of 126 ppm and conductivity of 0.35 mmhos/cm.

Anaerobic sediments were collected from 5-10 cm depth and had a reported *in situ* redox potential of -152 mV. Sediments were sieved (2 mm) prior to testing.

Portions of sediment (28–30 g) were combined with 100 mL portions of the corresponding surface water, vigorously mixed then stored for an equilibration period of 33 d in the dark at 20 ^{o}C. Following this period the headspace of the aerobic microcosms was exchanged with fresh air and the reaction mixtures were prepared. The viable test mixtures contained nominal HBCD at 4.31 mg/kg sediment dw. In addition, biologically inhibited control mixtures (heat sterilized), benzoate control mixtures (to quantify microbial activity) and toxicity control mixtures (benzoate + HBCD to evaluate the impact of HBCD on microbial activity) were maintained. The microcosms were incubated at 20±2^{o}C in the dark. Sediment microcosms were analysed on days 0, 5, 7, 14, 21, 28, 56, 84 and 113 and toxicity controls were analysed for $^{14}CO_2$ on days 7 and 15. The headspace gases were analysed for volatile ^{14}C-products.

The pH and redox potential of selected microcosms were regularly measured throughout the study. The pH ranged from 6.2 to 6.8 while the redox potential ranged from 24 to 88 mV at the bottom of the water layer. According to Tebbutt (1992), anaerobic reactions occur below +50 mV. Therefore these redox potentials may not represent truly anaerobic conditions, but are still likely to be reducing.

In the viable microcosms the total HBCD concentration decreased from 96% to 37% AR after 112 d with the following results obtained for the individual isomers:

Table A4.11. Recoveries of HBCD and individual isomers (%AR) over the study period

Day	0	5	7	14	21	28	56	84	112
A	9.0	9.2	8.3	8.2	8.1	8.5	6.0	5.7	4.4
B	8.0	8.1	7.9	7.2	6.2	6.3	3.3	1.8	1.4
Γ	78.6	74.2	71.7	63.4	51.6	51.3	39.0	30.0	31.2
TOTAL HBCD	95.6	91.5	88.0	78.8	65.9	66.1	48.2	37.5	37.0

Some loss was noted in the abiotic control with ^{14}C-HBCD decreasing from 112% to75% AR over the same time period.

Three degradation products were detected, and based on their retention times, were considered the same products I, II and III found in the sludge degradation studies. In the viable sediments, Product I reached a maximum of 20% after 28 d then decreased to 6% by day 113. Products II and III began to appear after 2–3 weeks and steadily increased to 45% and 10% respectively by the conclusion of the study.

Some loss of HBCD was found in the biologically inhibited controls with HBCD decreasing from around 112% AR at the start of the study to around 75% AR after 113 d. The appearance of degradation products were noted with Products I, II and III reaching maximum concentrations of 23%, 10% and 2% AR at the conclusion of the study.

There was no significant production of $^{14}CO_2$ or other volatile products as the measured concentration of radioactivity in the headspace gas was always <1% AR. Through the first 28 d, methane concentrations in the headspace ranged from 1%-2% in the viable sediments. Thereafter, methane concentrations continued to increase, reaching 20% after 84 d. By contrast, methane concentrations remained at around 0.2% AR in the

biologically inhibited controls. For both the viable and inhibited anaerobic microcosms, most of the radioactivity remained in the sediment layer with <2% typically found in the aqueous fraction.

The potential for HBCD to inhibit biological activity in the aquatic sediment was evaluated by examining the mineralization of ^{14}C-benzoate. Mineralisation of this substance incubated aerobically and anaerobically without HBCD reached around 12% and 34% after 28 d and 15 d respectively. Mineralisation of benzoate was also noted in the sediments amended with around 4 mg/L HBCD (toxicity controls) with $^{14}CO_2$ ranging from 21% (day 28) to 37% (day 15), indicating no inhibition of the sediment by HBCD.

The degradation half-life in anaerobic sediment was not determined in this study. However, plotting ln(concentration %AR) against time showed first order degradation kinetics ($r^2 = 0.94$). A rate constant was determined to be 0.0092 and a half-life (through combined biotic and abiotic routes) determined to be 75.3 d.

A4.1.2 Biotransformation

To test if biotransformation by the cytochrome P450 system could explain the observed compositional difference with technical HBCD mixtures (mainly γ-HBCD), a number of in vitro assays with microsomal preparations of liver were carried out (Zegers et al., 2005). Active proteins were taken from laboratory rats and harbour seals as substitutes for cetaceans. The peaks of the β and γ isomers of HBCD in an artificial 1:1:1 mixture of all 3 isomers showed a highly significant decrease (around 69% and 60% respectively) after 90 minutes and there were indications of metabolites formed. The α-isomer was not significantly biotransformed even after 90 minutes incubation at 37 °C. These assays showed that β- and γ-HBCD isomers were significantly metabolised when incubated in the presence of NADPH as an electron donor, compared to a set of reference samples which were identical except for the addition of NADPH. In contrast, the peak of α-HBCD did not decrease significantly in the presence of NADPH.

Law et al. (2006a) examined the bioisomerisation potential of HBCD in juvenile rainbow trout (*Oncorhynchus mykiss*). The investigation was performed in vivo allowing a concurrent examination of bioaccumulation parameters for individual isomers. The bioaccumulation results are discussed separately under "Bioaccumulation" below. Fish were exposed through the food and lipid corrected concentrations of α, β and γ diastereoisomers in the food were determined to be 29.14, 11.84 and 22.84 ng/g respectively. Respective lipid corrected concentrations of α and γ diastereoisomers in the control food were 0.07 and 0.30 ng/g while the β isomer was below the method detection limit. Fish (129 in total, initial mean weights of 233 g) were separated into 4 800 L tanks that were kept at a constant water flow rate of 1.5 L/min, with a temperature of 11 to 12 ^{o}C and a pH between 7.9 and 8.1.

Dissolved oxygen was always at a level of saturation. Fish were acclimatized in their respective tanks for 7 d. For each tank, fish were fed a different isomer fortified food with one tank receiving control food. Fish were fed 1% average total bodyweight of the tank 3 times a week. The amount of food was adjusted after each sampling day. The exposure (uptake) phase lasted 56 d and after this time, all fish were fed control food for a 112 d depuration phase. Muscle tissue of 4 fish from each tank was sampled on days 0, 7, 14 and 15 of the uptake phase and 0, 7, 14, 56 and 112 of the depuration phase.

The diastereoisomeric profiles of HBCD in the exposed fish were used to examine the possibility of bioisomerization. Metabolite formation in the liver and muscle mediated by P450 enzymes was also examined. Only muscle tissue from the carcass was used for calculating bioaccumulation parameters, whereas liver and muscle tissues were used for screening for phase I cytochrome P450 enzyme activities and debromination metabolites. Tissue analysis was done by LC coupled to tandem MS so that near baseline resolution of all 3 congeners was obtained.

Following the depuration period, fish exposed exclusively to the β isomer showed statistically significant molar amounts of the α isomer (p <0.01) and γ isomer (p <0.02) compared to the controls. This ability to bioisomerise along with the fact that the β isomer is present in small concentrations in the commercial HBCD mix helps explain why it is present in relatively smaller concentrations in biota.

Where fish were exposed to the α isomer, no β isomer was detected after the depuration period while a small amount (~0.1 nmol) of γ isomer was found at the end of the depuration period. However, where exposure was to the γ isomer, a linear increase in the α isomer was found over the first 14 d depuration (approaching 0.4 nmol after 14 d). This isomer was still found at around 0.2 nmol after 112 d depuration compared to around 0.18 nmol of the γ isomer at this time (note, values read from a graph). No β isomer was found after 112 d depuration and only very small amounts of this isomer were found at other sampling times. This finding indicates that trout have the ability to bioisomerise the β and γ isomers, but the α isomer is more resistant to bioisomerisation in this fish species.

Regarding other metabolites, no peaks from debrominated or OH-HBCD metabolites were found in the monitored ions of either the muscle or liver tissue extracts.

The authors conclude that, while it is unclear what enzyme system(s) is/are responsible for the observations, selective bioisomerisation appears to play a critical role in the isomer distribution of HBCD in environmental media.

A4.1.3 Bioaccumulation

Several studies were conducted to characterise bioaccumulation of HBCD in various species of animals, mostly aquatic. Results of these studies are discussed below.

Exposure through water

1. A flow-through bioconcentration test with the rainbow trout (*Oncorhynchus mykiss*) consisting of a 35 d uptake phase followed by a 35 d depuration phase was performed (Drottar & Krueger, 2000). The test was performed according to standardised guidelines (US EPA OPPTS 850.1730, ASTM Standard E1022-84, and OECD TG 305). The study was conducted in compliance with GLP standards. The test substance was a composite sample with purity of 90% and containing 6.4%, 5.4% and 79.1% α-, β- and γ-HBCD isomers respectively. Two exposure groups consisting of 0.34 and 3.4 µg HBCD/L in acetone and a solvent control group (acetone only) were maintained with 1 replicate per group. Eighty-five fish were used in each test group.

The test was performed using a continuous flow diluter with around 9.0 volume additions of test water delivered to test chambers every 24 h.

All fish were observed once each day to evaluate mortalities and sub-lethal behaviour. Water samples were taken at days –4 and –1 (pre-test period), 0 and 4 h (exposure period) and then at regular intervals during both the uptake and depuration periods. Tissue samples were collected on day 0 (4 h) and then at regular intervals during both the uptake and depuration periods. At each sampling time sufficient fish were collected to provide 2 replicate samples of solvent control fish and 4 replicate samples of each treatment group. Water and tissue samples were analysed for HBCD using LC/MS. The LOQ for water samples was 0.0250 µg HBCD/L. For the edible and non-edible tissues the LOQ was 1.0 µg HBCD/kg for uptake day 0 sample, 25.0 µg HBCD/kg for uptake days 1, 3 and 7 and depuration day 35 samples and 125 µg HBCD/kg for all other sampling times. While all tissue LOQs were based on the lowest calibration standard of 1 ppb HBCD, the difference in LOQs for tissues was due to different matrix blank dilution factors at the time of analysis.

Dissolved oxygen ranged from 8.8 to 10.6 mg/L with pH ranging from 7.9 to 8.3 over the course of the study. In the solvent control, the water had mean values for alkalinity of 178 mg $CaCO_3$/L, hardness of 137 mg $CaCO_3$/L and TOC of 10.4 mg C/L. No mortality or treatment-related clinical signs of toxicity were observed during the test.

In the low concentration group, the mean measured water concentration was 0.18 µg/L (53% of the nominal test concentration). Concentrations of HBCD during the depuration phase were generally <LOQ. Measured water concentrations of HBCD in the 3.4 µg/L treatment group ranged from 0.972 to 2.68 µg/L with an average concentration of 1.8 µg/L (53% of the nominal test concentration). Five of the 16 water samples analysed during the depuration phase contained measurable HBCD (0.029–0.3 µg/L). All other levels were <LOQ. While measured concentrations in both exposure groups were relatively consistent, they were still much lower than the nominal concentration.

Concentrations found in edible, non-edible and whole fish tissues during the uptake phase are shown (mean values of 4 replicates) in the following table.

Table A4.12. HBCD concentrations in fish tissues (µg/kg) during uptake phase

Time (days)	4 h	1	3	7	14	21	28	35
Low exposure group								
Edible	11.5	81.0	225	383	523	726	773	1173
Non edible	27.3	119	379	91	978	1942	2146	3731
Whole fish	18.9	98.1	289	628	747	1299	1498	2355
High exposure group								
Edible	109	705	2584	5145	6803	9831	8233	8613
Non edible	265	1200	4581	11 966	15 286	25 495	21 434	22 546
Whole fish	184	942	3414	8425	10 889	17 438	14 986	16 039

In the low exposure group, steady state was not reached. The BCF was therefore calculated on the basis of the day 35 values. In the high exposure group, steady state concentrations appeared to occur around day 21, although statistical analysis of variance showed that concentrations for uptake days 14 to 35 were not significantly different in edible tissues. BCF values for edible tissues in the high concentration group were based

on average values from days 14 to 35 while for non-edible tissues and whole fish, the BCF was calculated on average tissue concentrations for days 21 to 35.

BCF estimates were therefore calculated to be as follows.

Table A4.13. Water concentrations (µg/L), tissue concentrations (µg/kg) and estimated BCF values

	Low exposure group			High exposure group		
	Water conc.[1]	Tissue conc.[2]	BCF	Water conc.[1]	Tissue conc.	BCF
Edible	0.18	1175	6531	1.8	8370	4650
Non-edible	0.18	3731	20726	1.8	23 158	12 866
Whole	0.18	2355	13085	1.8	16 154	8794

1) Mean measured concentrations; 2) day 35 mean tissue concentrations.

The BCF values for the low exposure group may be underestimated, as no steady state was reached and even after the 35 d exposure period, concentration in all tissues were still showing a strongly linear increase ($r^2 = 0.96$ for whole fish tissue concentrations). Kinetic modelling was performed using BIOFAC. This model predicted much higher BCFs for the low exposure group of 14 039, 30 294 and 21 940 for edible, non-edible and whole fish tissues respectively. However, for the high exposure group where steady state did appear to be reached, this model predicted BCF values almost double those determined experimentally with BCFs of 9826, 23 303 and 16 450 for edible, non-edible and whole fish tissues respectively.

Table A4.14. HBCD concentrations in fish tissues (µg/kg) during depuration phase

Time (days)	1	10	14	21	28	35
Low exposure group						
Edible	1179	936	870	1314	517	617
Non-edible	2071	3366	2991	1728	1204	992
Whole	1658	2248	1918	1567	849	782
High exposure group						
Edible	9891	7992	8697	8723	1730	4013
Non-edible	23805	33723	17359	14171	7404	8324
Whole	17295	21336	12847	11359	4577	6449

In the low exposure group, levels found after up to 10 d of depuration in fish tissues were approximately the same as found in these tissues at the end of the uptake phase. From there, they slowly dissipated from the fish tissues over the remainder of the study. In the high exposure group, residues in all tissues at 1 d depuration were around the same as those at the end of the uptake phase. However, residues in the non-edible and whole fish tissues then continued to climb, peaking based on the sampling regime at 10 d into the depuration period. This is despite an apparent steady state being reached

during the uptake phase. From there, a slow decline in tissue levels was observed. If the peak depuration day 10 values were used to determine the BCF for the high exposure group, BCF values for non-edible and whole fish would be 18 735 and 11 853 L/kg respectively.

Kinetic modelling using BIOFAC has estimated depuration rate constants and clearance times for the 2 exposure groups as follows:

Table A4.15. Depuration rates for HBCD based on BIOFAC modelling

	Low exposure group			**High exposure group**		
	Dep. Rate constant (/d)	Time to reach 90% steady state (d)	Time to reach 50% clearance (d)	Dep. Rate constant (/day)	Time to reach 90% steady state (d)	Time to reach 50% clearance (d)
Edible	0.0196	118	35.4	0.0369	62.5	18.8
Non-edible	0.0232	99.3	29.9	0.0352	65.4	19.7
Whole	0.0228	101	30.4	0.0359	64.1	19.3

Exposure through food

2. Law et al. (2006a) examined the bioaccumulation parameters (depuration rates, half-life and BMF) of individual HBCD isomers in juvenile rainbow trout (*Oncorhynchus mykiss*). Fish were exposed to environmentally relevant doses of the 3 isomers separately through the diet with the test system described above (biotransformation). Bioaccumulation parameters were determined by analysis of muscle tissue concentrations.

None of the diasterioisomers reached steady state during the 56 d uptake phase. Uptake curves for both the α and β isomers increased exponentially with respective doubling times of 8.2 and 17.1 d respectively. Bioaccumulation of the γ isomer was linear, with a calculated uptake rate constant of 0.006/d.

Both the β and γ isomers followed first order depuration kinetics. The depuration of the α isomer showed an initial rapid depuration for the first 14 d followed by a slower depuration rate of the remainder of the experiment. Both the β and γ isomers were still accumulating after the first 7 d of depuration following which they slowly began to decline. The reason for this continuing accumulation early in the depuration phase is unclear, but it was hypothesised by the authors that assimilation of these isomers from the gut was slower than that of the α-HBCD isomer.

The following bioaccumulation parameters were found.

Table A4.16. Bioaccumulation parameters of HBCD diastereoisomers

	Depuration rate constant (k_d) (/d)	Half-life (d)	Average assimilation efficiency (%)	Bioaccumulation factor (BAF)
α-HBCD	Not determined	Not determined	31.1	9.2
β-HBCD	0.0044	157	41.4	4.3
γ-HBCD	0.0048	144	46.3	7.2

Based on the graphical values for depuration of α-HBCD in the report, the depuration half-life over the first 2 weeks of depuration can be estimated to be around 13 d. After that period, levels remained relatively constant during the remainder of the depuration phase.

Food web

3. As part of their BSEF study, de Boer et al. (2002) considered HBCD behaviour in various food chains.

North Sea food chain: Samples of animals representing different trophic levels (invertebrates, fish and sea mammals) were analysed to determine the environmental occurrence of HBCD. The model of the North Sea food chain comprised benthic invertebrates (sea stars, whelks and hermit crabs), fish (whiting) and sea mammals (harbour porpoises and harbour seals). From every trophic level in the North Sea food chain, a small number of samples were selected to investigate possible biomagnification. For the hermit crab, 9 samples were analysed to investigate the geographical distribution of the studied compounds.

The concentrations found (ng/g lw) are summarised as follows.

Table A4.17. Concentrations of total HBCD (ng/g lw) in biological samples

Species	Tissue sampled	No. samples	Concentration range	Mean concentration
Common whelk	Soft parts	3	29–47	35.3
Sea star	Pyloric caeca	3	<30–84	53.7
Hermit crab	Abdomen	9	<30	<30
Whiting	Fillet	3	<73	<73
Harbour porpoise	Liver/Blubber	5	729-6275	3079
Harbour seal	Liver/blubber	5	<36–2055	477

Some analysis of isomer specific concentrations was performed. In harbour porpoise, of the 5 samples tested, all showed the α isomer to be completely dominant in the congener profile (56%–97% of total HBCD). Similarly, for one harbour seal sample, the α congener was very dominant (71% total HBCD).

Tees food chain: The HBCD isomer profile was determined in cormorant livers (5 samples from 1999–2000). HBCD isomers were detected, but not all isomers in all

samples. The sum of the 3 isomers varied from 2.2 to 26.4 ng/g ww with a mean of 16 ng/g ww. The α isomer dominated, ranging from 70% to 100% of total HBCD levels.

Five samples of porpoise blubber (1998 specimens) were analysed. All were females. HBCD was found in 4 of the 5 samples, and total HBCD levels (where found) ranged from 54.4 to 917 ng/g ww. In 3 of these samples, the α isomer dominated (~98%–99% total HBCD levels). However, in one sample, the 3 isomers each accounted for around one-third of total levels. This was in the porpoise with the highest total HBCD levels.

Three samples (2001) of whiting muscle showed various results. HBCD was not detected in one sample, and was found at 290.9 and 1036 ng/g ww in the other 2 samples. In the sample with the highest levels, the α isomer accounted for around half the total levels, with the β and γ isomers each at around 25% total levels. In the other sample, the γ isomer was dominant (~51% total levels) with α and β isomers being present at similar levels. Lower levels of HBCD were found in one whole starfish collected in 2001. Of the total HBCD (16.92 ng/g ww), 10.2 µg/kg ww was α-HBCD and 6.72 ng/g ww was γ-HBCD. No β-HBCD was found.

Western Scheldt food chain: A sample selection comprising common tern eggs, gudgeon and mysid shrimp (from 2000 and 2001 sampling) were analysed for total HBCD and separate isomers. HBCD was not found in mysid shrimp. The gudgeon had a total HBCD concentration of 49 ng/g ww (230 ng/g lw). The HBCD concentrations in common tern eggs ranged between 35 and 640 ng/g ww (mean, 87 ng/g ww), and between 330 and 7200 ng/g lw (mean, 930 ng/g lw). The data confirm the relatively strong bioaccumulation potential of HBCD. The authors state that biomagnification seems to occur from the mysid shrimp, via the gudgeon to the terns. However, the numbers of mysid shrimp and gudgeon samples were too small to give reliable estimates of BMF. It is stated (although values did not seem to be in the report) that, in gudgeon, the main isomer was γ-HBCD. In the common tern eggs, α-HBCD was strongly dominating. In most samples the ratio α/γ-HBCD was substantially higher than 20, indicating biotransformation of γ- to α-HBCD in biota.

4. The biomagnification of α-, ß- and γ-HBCD congeners in a pelagic Lake Ontario food web has been studied (Tomy et al., 2004). Samples consisted of lake trout (*Salvelinus namaycush*, n = 5 whole fish, top predator fish) and the following forage fish species: alewife (*Alosa pseudoharengus*, n = 3 composite samples from 5 fish), rainbow smelt (*Osmerus mordax* n = 3 composites of 5 or 20 fish), slimy sculpin (*Cottus cognatus*, n = 3 composites of 10 or 15 fish). Invertebrate samples included mysids (*Mysis relicta*, n = 2 composites of >100 individuals) and amphipods (*Diporeia hoyi*, n = 2 composites of >100 individuals).

Samples were analysed by liquid chromatography tandem mass spectrometry (LC/MS/MS). The ß-HBCD isomer was below method detection limits in all samples. Concentrations (ng/g, ww) of α- and γ-HBCD in biota were measured and were detected in all food web samples (3 trophic levels) from Lake Ontario. α- and γ-HBCD levels were highest in the top predator lake trout samples: 0.4–3.8 ng/g ww for the α isomer and 0.1–0.8 ng/g ww for the γ isomer. For the prey (forage) fish species, the trends in α- and γ-HBCD levels were slimy sculpin > smelt > alewife. Mean concentrations of total HBCD (α- and γ-HBCD) in the macrozooplankton *Mysis relicta* and in the benthic invertebrate *Diporeia hoyi* were similar and approximately twice (based on reading from a graph) as high as in plankton.

There was a clear difference in the relative abundance of the α- and γ-HBCD isomers among the species. Expressed numerically as the concentration of the α isomer in the

samples divided by the sum of α and γ isomers, the fraction of α-HBCD in plankton, *Mysis* and *Diporeia* are 0.49, 0.78 and 0.69 respectively. For the forage fish, respective fractional amounts of the α isomer are 0.88, 0.76 and 0.86 for alewife, sculpin and smelt respectively, while, in lake trout, the α isomer accounts for 82% of the total. These values suggest an enrichment of α-HBCD at higher trophic levels.

There was linear ($r^2 = 0.72$, $p<0.0001$) relationship between the total HBCD concentrations and trophic level indicating biomagnification of HBCD in the Lake Ontario food web. The TMF was calculated to be 6.3, derived from the slope of total HBCD to trophic level relationship. This TMF was higher than for p,p'-DDE (6.1), and for sum of PCBs (5.7) also calculated in this paper.

Predator/prey biomagnification factors (BMF) for both isomers were >1, suggesting biomagnification between trophic levels. Lipid normalised BMFs for the α- and γ-HBCD isomers are shown below.

Table A4.18. Lipid normalized BMFs for α- and γ-HBCD, in a pelagic Lake Ontario food web

Predator/prey	α–HBCD	γ–HBCD
Trout/alewife	4.8	7.5
Trout/smelt	1.0	1.5
Trout/sculpin	1.1	0.8
Sculpin/*Diporeia*	3.5	2.5
Sculpin/*Mysis*	9.7	9.9
Smelt/*Mysis*	10.8	5.5
Smelt/*Diporeia*	4.0	1.4
Alewife/plankton	0.4	0.2

The highest levels found for sculpin and smelt to *Mysis* may be driven by the low lipid normalized HBCD concentrations in *Mysis*.

5. Leonards et al. (2004) describe data relating to the transfer of HBCD in 2 food chains (common tern and harbour seal) from the Dutch marine environment. Samples of sediment, suspended particulate matter (SPM), invertebrates (e.g. bivalves, shrimps, worms), and fish (e.g. sandeel, flounder, sole, goby, herring, whiting) were collected in the spring of 2003 in the feeding habitats of the tern (Western Scheldt estuary) and seals (Wadden Sea). Tern eggs were collected, in the same period, from the Terneuzen colony. Blubber samples of adult male harbour seals were collected from stranded animals from the Wadden Sea population. The invertebrates were placed, after collection, for 1 d in surface water to remove most of the sediment particles from the intestine. Whole animals were homogenized using a blender. Extracts were analysed for HBCD with GC/MS in the negative chemical ionisation mode, and with LC/MS using electrospray ionisation for the analysis of the isomeric composition of HBCD. HBCD levels increased from invertebrates to fish but decreased from fish to tern egg. This is suggested to imply that tern metabolises HBCD. Actual mean concentrations (as read from a graph) were in the order of 100 ng/g lw in invertebrates, 375 ng/g lw in sandeel and 225 ng/g lw in tern eggs. In the tern egg and fish samples, α-HBCD dominated

while the γ-HBCD dominated in the sediment and SPM samples (actual figures not provided).

6. Work on biomagnification potential in an arctic marine food chain is reported by Sørmo et al. (2006). Concentrations of HBCD were investigated in a food chain consisting of 4 invertebrate species: polar cod (*Boreogadus saida*), ringed seals (*Pusa hispida*) and polar bears (*Ursus maritimus*). Lower trophic sampling of the marine food web included 4 invertebrate groups: pelagic, herbivorous calanoid copepods (mainly *Calanus glacialis*); the herbivorous, pelagic krill *Thysanoessa inermis*; the pelagic omnivorous amphipod *Themisto libellula* and the ice-associated, omnivorous amphipod *Gammarus wilkitzkii*. All animals except one polar bear were collected between February and September 2003. The following levels were reported.

Table A4.19. HBCD concentrations (ng/g lw) in components of an Arctic marine food web

Species	*n*	Min	Max	Mean
Calanoid copepods	1	ND		
T. inermis	1	ND		
T. libellula	1	ND		
G. wilkitzkii	1	NA		
Polar cod	7	1.38	2.87	1.89
Ringed seal[1]	6	14.6	34.5	19.56
Polar bear[2]	4	20.74	44.55	30.04

1) Blubber; 2) Adipose tissue.

Based on mean lipid weight findings, HBCD appears to biomagnify from polar cod to ringed seal (BMF = 10.3), with a slight biomagnification from ringed seal to polar bear ((BMF = 1.5).

7. Law et al. (2006d) examined the extent of contamination and bioaccumulation of BFRs in a Lake Winnipeg food web. Fish samples consisting of Walleye (*Stizostedion vitreum*) whitefish (*Coregonus clupeaformis*), emerald shiner (*Notropis atherinoides*), burbot (*Lota lota*), white sucker (*Catostomus commersoni*), and goldeye (*Hiodon alosoides*) were collected in the south basin of Lake Winnipeg on the Canadian Coast Guard Namao between 2000 and 2002. Mussels (*Lampsilis radiate*) were collected in 2002 (muscle tissue was removed for analysis, so depuration was not an issue). Net plankton samples containing a mixture of phytoplankton and zooplankton were also collected for analysis. The following results were reported:

Table A4.20. Mean HBCD concentrations (ng/g lw) in fish, sediment and water from the south basin of Lake Winnipeg

		Isomer		
Species	***n***	**α**	**β**	**γ**
Fish				
Walleye	5	6.53	1.26	4.20
Whitefish	5	1.22	0.53	1.03
Emerald shiner	5	8.25	1.11	15.32
Goldeye	3	8.64	0.85	1.39
White sucker	5	8.70	1.65	6.95
Burbot	5	8.58	1.09	4.96
Mussels	5	3.66	0.56	3.94
Zooplankton	5	15.71	5.58	44.13
Sediment	4	ND	ND	0.05
Water (pg/L)		10.63	ND	2.89

ND: Not Detected

The report calculates predator/prey BMF, and these are >1 for several pathways for the various HBCD isomers, with lipid-adjusted BMFs calculated as follows.

Table A4.21. Lipid-adjusted biomagnification factors for HBCD isomers

	Isomer		
Predator/prey	**α**	**β**	**γ**
Walleye/emerald shiner	1.1	0.8	0.6
Walleye/white suckers	1.8	2.2	1.1
Walleye/white fish	5.3	2.4	4.1
Walleye/goldeye	0.8	1.1	0.8
Emerald shiner/zooplankton	0.7	1.9	5.0
White suckers/zooplankton	0.4	0.6	2.8
White suckers/mussels	0.4	0.5	0.3
Burbot/emerald shiner	2.7	3.4	6.3
Burbot/mussels	1.9	5.0	2.9
White fish/zooplankton	0.1	0.6	0.9
White fish/emerald shiner	0.2	0.3	0.1
Goldeye/zooplankton	1.0	1.3	3.6
Goldeye/mussels	8.2	1.0	0.3

In addition, TMFs were calculated to assess the food-web magnification for the entire food web based on the relationship between trophic level (TL) and contaminant concentration. The rank order of TLs were mussel → zooplankton, white fish → goldeye, white sucker → burbot, walleye (top predators). A significant relationship was found for γ-HBCD, suggesting that HBCD is biomagnifying within the food web. The calculated TMF for ∑HBCDs in Lake Winnipeg was 3.1 (2.3, 2.3 and 4.8 for the individual α, β and γ isomers respectively).

8. Jenssen et al. (2007) undertook a separate study to characterise exposure of HBCD in animals from different trophic levels in North-East Atlantic coastal marine ecosystems along a latitudinal gradient from southern Norway to Spitsbergen, Svalbard, in the Arctic. Calanoid species were collected from the Oslofjord (59°N), Froan (64°N), and Spitsbergen (>78°N); Atlantic cod (*Gadus morhua*) from the Oslofjord and Froan; polar cod (*Boreogadus saida*) from Bear Island (74°N) and Spitsbergen; harbour seal (*Phoca vitulina*) from the Oslofjord, Froan, and Spitsbergen; and ringed seal (*Pusa hispida*) from Spitsbergen. Eggs of common tern (*Sterna hirundo*) were collected from the Oslofjord, and eggs of Arctic terns (*Sterna paradisaea*) from Froan and Spitsbergen.

The following results are reported.

Table A4.22. Mean concentrations (ng/g lw) in species from different trophic levels n marine coastal ecosystems in the Norwegian North-East Atlantic

Location/species	∑HBCD
Oslofjord	
Calanus (sp)	4.01
Atlantic cod	25.6
Harbour seal	50.5
Common tern	36.4
Froan	
Calanus finmarchicus	-
Atlantic cod	18.7
Harbour seal	22.3
Arctic tern	17.0
Bear Island	
Polar cod	11.7
Spitsbergen	
Calanus glacialis	-
Polar cod	1.80
Harbour seal	3.66
Ringed seal	19.6
Arctic tern	4.62

Levels of HBCD generally decreased as a function of increasing latitude, reflecting distance from release sources. HBCD was found in animals from all trophic levels, except in calanoids at Froan and Spitsbergen. HBCD was biomagnified from cod to harbour seals at all 3 locations (BMF values of around 1.2–2.0 based on mean concentrations).

9. The trophodynamics of bromine-based flame retardants were examined in components of a marine food web from the western Canadian Arctic (Tomy et al., 2009). The animals studied and their relative trophic status in the food web established (using stable isotopes of nitrogen [δ15N]), were beluga whale (*Delphinapterus leucas*) > ringed seal (*Pusa hispida*) > Arctic cod (*Boreogadus saida*) > Pacific herring (*Clupea pallasi*) ≈ Arctic cisco (*Coregonus autumnalis*) > pelagic amphipod (*Themisto libellula*) > Arctic copepod (*Calanus hyperboreus*). For HBCD, the lipid adjusted concentrations were reported as follows.

Table A4.23. Mean concentrations of HBCD isomers (ng/g lw) in components of a Western Arctic marine food web

		Congener			
Species	***n***	**α**	**β**	**γ**	**∑HBCD (mean)**
Beluga	10	0.53–2.25	0.01–0.03	0.01–1.32	1.48
Ringed seal	8	0.01–2.42	0.01–0.24	0.01–1.59	0.66
Arctic cod	9	0.03–12.33	0.01–4.96	0.01–26.56	6.09
Pacific herring	10	0.03–2.27	0.01–0.97	0.01–4.54	1.06
Cisco	9	0.03–3.78	0.01–2.43	0.01–11.15	0.99

There were differences in the concentration profiles of the isomers of HBCD in the food web. The most notable difference was observed for beluga, where the α isomer was enriched (accounting for ~90% of the ∑HBCD body burden) relative to its primary prey species, Arctic cod, where the α isomer accounted for only 20% of the ∑HBCD body burden (β: 4% and γ: 78%).

10. HBCD was also considered in an earlier study assessing potential for trophic transfer in a marine food web from the eastern Canadian Arctic (Tomy et al., 2008). The following results were noted in various species of animals arranged in the order of highest trophic level to lowest trophic level.

Table A4.24. Mean concentrations (ng/g lw) in components of an eastern Arctic marine food web

		Congener		
Species	***n***	**α**	**β**	**γ**
Narwhal	5	2.05–6.10	ND	<0.11–1.27
Beluga	5	<0.63–2.08	ND	<0.07–0.46
Walrus	5	ND–0.86	ND	<0.12–1.86
Arctic cod	8	ND–1.38	ND	ND–0.07
Red fish	5	<0.74–3.37	ND	<0.28–1.03
Clam	5	ND–1.03	ND	<0.46–5.66
Shrimp	5	0.91–2.60	ND	0.23–1.24
Zooplankton	5	ND–9.16	ND	0.13–2.66

ND: Not Detected

The authors calculate positive biomagnification (BMF >1) for α-HBCD from cod to narwhal (BMF = 4), and cod to beluga (BMF = 2). Positive BMF for the γ-HBCD isomer are calculated for cod to narwhal (BMF = 17) and cod to beluga (BMF = 7). Regarding trophic magnification, α-HBCD showed an increase in concentration with increasing trophic level, and a TMF of 2.1 was calculated. Conversely, there was trophic dilution of γ-HBCD with a TMF of 0.5 calculated, possibly due to conversion of this isomer within biota.

A4.1.4 Measured levels in biota

Australian data

No Australian monitoring data are available.

International data

Aquatic

1. Sellström et al. (1998) report levels of HBCD in fish from a Swedish river. Pike were collected from 4 sites in the River Viskan, and from one location in a nearby water system in 1995. Analysis was performed using gas chromatography/mass spectrometry with HBCD scanned in the ECNI mode. No recovery experiments were performed for HBCD in fish, so results are semi-quantitative – that is, no correction for recovery has been made. At one upstream site, 3 fish samples all had HBCD levels of <100 ng/g lw. At the nearby water system, 3 fish samples had levels ranging from <50–<90 ng/g lw. Site 2 was downstream from 1 textile operation and levels were low at <100 ng/g (4 samples). However, sites 3 and 4 (downstream from 1 STP and 2 and 3 textile industries respectively) had elevated levels of 4 000–8 000 ng/g lw (3 and 2 samples at sites 3 and 4 respectively).

2. Nylund et al. (2001) report total HBCD levels in herring (*Clupea harengus*) from the Baltic Sea. Twelve specimens were collected and analysed from 5 different sites. All sites were considered reference sites with no known local sources of pollutants. Sampling was conducted in autumn of 1999 and all fish specimens were females between 2 and 4 years old. Muscle samples were extracted and analysed by GC/MS. In general, significantly higher concentrations were found for HBCD in the samples from the southern Baltic Proper compared to the other sites. This could not be explained by an extremely low fat content or by any deviant biological variables such as age or size. The range of HBCD concentrations in this area was 28–36 ng/g lw (mean, 32 ng/g fat). At the other 4 sites, mean values ranged from 4.9–9.8 ng/g lw, with individual samples ranging from 4.3–13 ng/g lw.

3. Results from further 2002 sampling of herring at 6 different sites along the Swedish coast are reported (Asplund et al., 2004). Again, all specimens are reported as females between 2 and 4 years of age. The geometric mean concentrations of HBCD ranged from 3.98–18.3 ng/g lw. The range of individual samples was 1.5–31 ng/g lw. This paper compared concentrations found in the herring samples from 1999 to 2002 and concluded that HBCD showed a strong regional differentiation, with higher levels in the southern Baltic Sea that may indicate ongoing inputs to this part of the region. Over this small 4-year period, in all but one sampling region, there did not appear to be any increasing trend in concentrations. One sampling area (Landsort) showed a slight increase in muscle concentrations from around 10 ng/g lw in 1999 to around 13–14 ng/g lw in 2002 (values being mean concentrations and read from a graph).

4. Schlabach et al. (2004) focused on the pollution of BFRs in Lake Mjøsa, where unusually high concentrations of PBDEs have been previously found in fish. The objective of the study described was to make a broader documentation of the levels in sediments and fish, and to localise areas with point sources. Lake Mjøsa is situated in Southeast Norway and is the country's largest lake (365 km^2, average depth 153 m, maximum depth 449 m). Surface sediment samples (0–2 cm) were collected from 14 stations. One sediment sample was also collected from the main inlet river at Lake Losna, 25 km north of Lake Mjøsa. Fish were collected from the inlet river at Lake Losna, from Lake Mjøsa, from the outlet river Vorma, and from Lake Øyeren further down the watercourse. Fish samples were homogenates of whole body or muscle fillets (pooled samples, typically 7–20 individuals per sample). The indicator species were brown trout (*Salmo trutta*), perch (*Perca fluviatilis*), pike (*Esox lucius*), burbot (*Lota lota*), vendace (*Coregonus albula*) and smelt (*Osmerus eperlanus*). Archived samples of vendace fished during 1993–2002 were also included. Extracts were analysed with LC/MS for the analysis of the isomeric composition of HBCD. Of the sediment samples from Lake Mjøsa and Lake Losna, the only detectable level was found outside the town of Lillehammer in the northern part of Lake Mjøsa. Only β-HBCD was found here, with a concentration of 7.9 ng/g dw. HBCD was found in fish from Lake Mjøsa and further down the watercourse in the river Vorma and Lake Øyeren. The levels in fish were in the range of 90–880 ng/g lw. The α isomer dominated the concentrations. The highest level was found in a pike sample from Lake Mjøsa.

5. Bytingsvik et al. (2004) report BFR concentrations in cod (i.e. Atlantic cod (*Gadus morhua*) and polar cod (*Boreogadus saida*)) at different locations in Norwegian waters, ranging from the estuary of the largest river in Norway, Glomma, to the pristine Arctic waters of Svalbard. Glomma has its outlet near Hvaler, outer Oslofjord, and is draining water from some of the largest rivers and lakes of the more industrialised areas of Norway. Concentrations of BFRs in Atlantic cod sampled at Hvaler (58°59'N, 10°47'E) were compared to concentrations in Atlantic cod sampled at Froan, (64°10'N, 09°20'E) situated in the more open waters of the Norwegian Sea off coast of Mid-Norway and polar cod from Bear Island, Svalbard (73°4'N, 18°3'E). A second aim was to study temporal changes in concentrations of BFRs in Atlantic cod from Hvaler in the period 1998–2003.

Atlantic cod were collected at Hvaler and Froan in June 2003 using fishing rods. Benthic trawl was used to collect Atlantic cod at Hvaler in April 1998 and polar cod at Bear Island in September 2003. The lipid content found in Atlantic cod sampled at Hvaler in 1998 ranged from 5%–20% and at Hvaler and Froan in 2003 the lipid content in Atlantic cod ranged from 8%–48%, whereas the lipid content in polar cod from Bear Island ranged from 5%–25 %. The weight and length of Atlantic cod ranged from 220–991 g and 28–48 cm, respectively, whereas the weight and length of polar cod ranged from 153–373 g and 27–36 cm, respectively. Both female and male Atlantic cod were collected at Hvaler in 1998, while only female Atlantic cod were collected at Hvaler and Froan in 2003. The sex of the polar cod from Bear Island 2003 was unknown.

The samples were homogenized and the BFRs, including HBCD, were quantified using GC/MS. Mann-Whitney test was used to test differences in concentrations between the individual sites and between the 1998 and 2003 samples from Hvaler. A significance level of $\alpha = 0.05$ was chosen.

Spatial trends of BFRs: HBCD was found in highest concentrations in the cod from Hvaler (nd–56.9 ng/g lw), followed by cod from Froan (nd–51.2 ng/g lw) and Bear Island (7.67–23.4 ng/g lw).

Temporal trends of BFRs in Atlantic cod at Hvaler from 1998 to 2003: When comparing Atlantic cod from Hvaler in 1998 and 2003, the concentration of HBCD was about 8 times higher in 2003 as compared to 1998 in terms of wet weight, but, on a lipid weight basis, HBCD showed an increase of 3–4 times. This study indicates that there has been an increase in concentrations of both PBDEs and especially HBCD in the Hvaler and outer Oslo fjord area during the last 5 years. A geographical trend was found comparing the 3 localities – Hvaler, Froan and Bear Island – with decreasing concentrations from south to north. The most apparent increase was found from Hvaler and Froan compared to Bear Island. This study points out that the concentration of HBCD, in particular, has been increasing in the last few years, and that HBCD is now present in relatively high concentrations even in remote areas like Bear Island.

6. As part of a wider study considering HBCD in the Swedish environment, Remberger et al. (2004) report on fish levels (pike and eel) taken both upstream and downstream from an STP treating effluent from a textile industry in the river Viskan. The fish showed significantly higher HBCD levels downstream from the STP than upstream. The levels varied between 65 and 1808 ng/g lw.

7. Gerecke et al. (2003) reported different HBCD isomer concentrations in Whitefish (*Coregonus sp.*) samples from 6 Swiss lakes. Samples consisted of pooled fillets from 10 fish. Total HBCD concentrations were determined by GC/MS analysis with isomers quantified using LC/MS. The recovery for the analytical procedure was around 62% and it is unclear whether the results reported have been corrected for recovery. The total HBCD concentrations in the whitefish ranged from 25 to 210 ng/g lw. The isomer distribution was quantified for samples of fish from 4 of the 6 lakes. The α-HBCD isomer dominated and was >85% total HBCD from 3 samples and >58% in the other lake.

8. A Bromine Science and Environmental Forum (BSEF) study on the occurrence of HBCD in the environment (sediments, biota and sewage sludge) has been performed (de Boer et al., 2002). The objectives of the study with respect to HBCD included analysing concentrations in:

- eel and sediments from the Scheldt Basin (Belgium)
- sewage sludges and landfill leachates from the Netherlands, the UK and Ireland
- sediments and biota from The Netherlands, the UK and Ireland, including the North Sea.

To avoid potential thermal rearrangements and co-elution during analysis with GC, a LC/MS method was developed, which also included isomer specific determination of HBCD. The results of this study were later published (Morris et al., 2004).

Total HBCD in eel, Scheldt Basin: In total, 18 eel samples (yellow eel, *Anguilla anguilla*) were taken during sampling campaigns in 2000. The samples are taken from the Scheldt Basin but also include 3 samples from reference sites. Sediment samples were obtained through the same institute from the same locations but sampled in 2001. Samples from 2000 were not available and new eel samples would only be sampled again in 2002. It was expected that differences in environmental conditions over a period of 1 year would be relatively small. However, the occurrence of incidental variations should be taken into account.

The highest HBCD concentrations in eels were found in the Scheldt near Oudenaarde (33 000 ng/g lw), Leie St Martens (7100 ng/g lw), Leie Oeselgem (4700 ng/g lw) and Dender, Appels (1300 ng/g lw). The HBCD concentrations in and around Antwerp

harbour were generally lower. In 2 of the 3 reference samples HBCD was found: 210 ng/g lw in eel from the Yzer near Nieuwpoort and 32 ng/g lw in eel from Achelse Kluis. These results show that HBCD contamination is to some extent independent of sources. HBCD sources can be expected to be present at the Scheldt near or upstream from Oudenaarde and at the Leie near St Martens and Oeselgem. More upstream in the Leie, near Wevelgem and Wervik, substantially lower HBCD concentrations were found.

In eel, apart from a number of non-detects, the α/γ-HBCD ratios were around 2.5–3.5. However, at the location Scheldt, Oudenaarde, a deviating pattern was found, with an α:β:γ ratio 21:21:1. This is interesting, because this was the location with the highest total HBCD concentration in eel. At the other location with a relatively high total-HBCD – Leie, Oeselgem – β-HBCD was also found, ratio α:β:γ: 5:1:2.

In most other eel samples β-HBCD was not found. γ-HBCD was also found in eel from the Leie, St Martens, and in eel from the Scheldt near Doel. In none of the samples was β-HBCD or γ-HBCD higher than α-HBCD.

Dutch rivers: Eleven eel samples from various Dutch rivers from 1999 (one sample from 2001) showed total HBCD levels ranging from 2.3 to 100 ng/g ww (12–570 ng/g lw). The results suggested that the HBCD found originated from Germany or further upstream the River Rhine.

Cod liver: HBCD was only found in the cod liver sample from the Central North Sea at a level of 50 ng/g lw. No HBCD was found in the southern North Sea sample. Also, in hake liver from the Atlantic, south-west from Ireland, HBCD could not be found. The isomeric profile of HBCD in cod liver from the Central North Sea only showed α-HBCD.

Concentrations of BDE-209 (along with other contaminants) were evaluated in archived Lake Ontario, Canada, lake trout (*Salvelinus namaycush*) samples collected between 1979 and 2004 (Ismail et al., 2009). Trout 4 to 5 years old were collected every 4 to 6 years (1979, 1983, 1988, 1993, 1998, and 2004), with 5 individuals (4 in 1979) per time point.

Table A4.25. Mean concentrations (ng/g lw) in Lake Ontario, Canada, lake trout

	Congener			
Year	**α**	**β**	**Γ**	**∑HBCD**
1979	25	0.94	6.5	33
1983	25	0.40	2.9	28
1988	15	0.26	2.5	18
1993	27	0.38	4.6	32
1998	22	0.28	2.3	25
2004	15	0.16	1.4	16

Total levels of HBCD showed a significant, exponential and declining trend during the sampling years. The half-life was in the region of 35 years, although the data were not well correlated (r^2 ~0.31 based on ln transformed mean concentration data). α-HBCD was the dominant isomer. This was also the most recalcitrant in terms of declining levels over the years of sampling, with a half-life in levels from the sampled trout of ~51 years (r^2 = 0.23), compared to the low levels of β-HBCD ("half-life" ~13 years, r^2 =

0.73) and γ-HBCD ("half-life" ~16 years, $r^2 = 0.58$). The slower rate for α-HBCD may be the result of bioisomerisation of the other 2 isomers, but this is unclear.

9. Shaw et al. (2009) undertook a study to determine concentrations of HBCD in species of teleost fishes that are components of the harbour seal diet. While for the wider study 7 species of fish were sampled, for HBCD, only 3 species were analysed, being Atlantic herring (n = 20), Alewife (n = 10) and Atlantic mackerel (n = 10). HBCD was detected in 87% of the fish samples at concentrations ranging from 2.4 to 38.1 ng/g lw. Mean total HBCD concentrations for these fish were 23, 7.6 and 14 ng/g lw respectively.

Marine mammals

1. Roos et al. (2001) report levels of total HBCD in blubber of juvenile male grey seals from the Baltic. Blubber from 20 young grey seals was collected between 1980 and 2000 and analysed for PBDEs and HBCD. The seals were between 1 and 3 years old. They were divided into 3 groups: seals collected between 1980 and 1985 (healthy), seals with no or slight intestinal ulcers (1993–2000) and seals with moderate, severe or fatal intestinal ulcers (1993–2000). Samples were analysed by GC/MS. Differences were found for HBCD ($p<0.002$), indicating higher concentrations in seals from the 1990s than in the 1980–85 sample group. Concentrations of HBCD ranged from 16 to 177 ng/g lw, with a median concentration of 59 ng/g lw. Similarly, statistically significantly higher levels of BDE-153 and BDE-154 were found in the 2 groups from the 1990s, and apart from these higher concentrations, no other obvious differences in concentrations were found between the 3 groups that could be connected with the disease. However, there may be several other chemical contaminants responsible or contributing to intestinal ulcers that were not determined for as part of this study.

2. Stapleton et al. (2006) considered the concentrations of BFRs, including HBCD, in stranded sea lions in California to determine if concentrations had changed over time. Blubber samples from 25 male California sea lions were selected for analysis, with all samples collected by the Marine Mammal Centre in Sausalito, California. The reported concentrations are in terms of total HBCD. HBCD was detected in 81% of the samples, and, where found, ranged from 0.3 to 11.9 ng/g ww (0.9–96.4 ng/g lw). The following results were reported.

Table A4.26. Mean levels in Californian sea lions

Year	1993	1997	1997	1998	1999	2000	2002	2003
No. samples	1	7	8	1	2	4	2	1
ng/g lw	5.30	3.46	4.30	1.10	4.80	30.3	10.8	17.2
ng/g ww	0.71	0.61	1.95	0.63	3.33	4.92	7.12	11.8

In terms of HBCD levels in wet weight, a relatively strong linear time trend was observed, with increasing mean concentrations (0.71 ng/g ww in 1996 to 11.9 ng/g ww in 2003; $r^2 = 0.76$). However, when the values were corrected for lipid weight, the relationship for increasing concentrations over time was not strong ($r^2 = 0.28$). While separate isomer values were not provided in the report, the authors state that α-HBCD was the dominant isomer detected in all samples and ranged from 62% to 100% total HBCD levels.

3. Law et al. (2006b) report the concentrations of HBCD in the blubber of harbour porpoises stranded or by-caught in the UK between 1994 and 2003. Eighty-five samples were analysed for HBCD, with analysis undertaken using LC/MS on a diastereoismer basis.

Table A4.27. Individual isomer ranges, total HBCD ranges and mean total HBCD values from various sampling years (ng/g ww)

Year	No. Samples	α-	β-	γ-	Total HBCD	Mean total HBCD
1994–99	23	10–460	<5–23	<5–10	10–470	115
2000	13	39–230	<3–18	<4–13	39–260	150
2001	15	103–10900	<4–37	<4–21	125–10 960	1400
2002	18	84–18400	<5–37	<5–<20	93–18 430	4600
2003	16	132–19200	<4–54	<5–21	132–19 208	7420

The results show that α-HBCD dominates over the other isomers. It was detected in all samples analysed. The time trend indicates a sharp increase in HBCD concentrations from 2001 onwards. Mean levels of total HBCD show a strongly linear correlation (r^2 = 0.97) in increase with mean levels increasing from 2000–03 by around 50 times.

4. Since this study, Law et al. (2008) have presented data for an additional 138 porpoises which extend the timeline to 2006. The data comprises 16 porpoises from 2003, 31 from 2004, 63 from 2005, and 28 from 2006. The β and γ isomers were rarely found, and the following results for α-HBCD and mean ∑HBCD results were reported.

Table A4.28. Individual isomer ranges, total HBCD ranges and mean total HBCD values from various sampling years (ng/g lw)

		α-HBCD		
Year	No. Samples	Min	Max	Mean total HBCD
2003	16	72	11 500	5450
2004	31	19	4150	1360
2005	63	<5	8470	1180
2006	28	64	6358	817

While investigation of time trends in the previous study confirmed a statistically significant increase between 2000 and 2001, this study confirms a statistically significant decrease between 2003 and 2004, and the data show mean levels continuing to fall through to the 2006 samples. Neither trend was confounded by age, sex, nutritional status, or location. The authors theorise that possible contributory factors to the observed decrease include the closure in 2003 of an HBCD manufacturing plant in north-east England, which had considerable emissions up to 2003, and 2 voluntary schemes intended to reduce emissions of HBCD to the environment from industry which, however, did not formally begin until 2006.

5. Total HBCD levels were measured in blubber of harbour porpoise (*Phocoena phocoena*) and common dolphin (*Delphinus delphis*) stranded on the west and east coasts of Scotland, the east, south, and west coasts of Ireland, the coasts of The Netherlands, Belgium and France north of Calais (Southern North Sea), the French coast of the western part of the English Channel, the Bay of Biscay and the north and west

coasts of Galicia in Spain (Zegers et al., 2005). Only female animals were chosen to limit the variation in levels from sex differences. Moreover, female harbour porpoises migrate less than males in these waters so are considered more representative for the area. Analysis of total HBCD was carried out using GC combined with negative chemical ionization mass spectrometry. Diastereomeric composition of a selection of blubber samples was determined using LC/MS.

The highest total HBCD levels were measured in harbour porpoises stranded on the Irish and Scottish coasts of the Irish Sea (medium concentration 2900 ng/g lw) and the northwest coast of Scotland (median concentration of 5100 ng/g lw). These levels were significantly higher than the levels in all other areas. The isomer composition of HBCD residue in blubber of a selection of 10 harbour porpoises and 9 common dolphins showed samples to contain exclusively the α isomer. Raw data are not provided with the paper or supporting information.

6. Peck et al. (2008) consider levels of HBCD in blubber (n = 57) and liver (n = 16) samples from Atlantic white-sided dolphins (*Lagenorhynchus acutus*). Concentrations of α-, β-, and γ-HBCD and their enantiomeric distribution in the tissues of these dolphins were evaluated, and the temporal trend of concentrations assessed. All samples were collected from animals stranded in good condition on the eastern United States coast from 1993 to 2004. While α-HBCD was quantified in all blubber and liver samples, the other 2 isomers were not detected. The samples came from both male and female animals and, in this study, while adult females did have lower mean concentrations than adult males and juveniles, this difference was not significant and the following results for α-HBCD are reported based on both sexes and all life stages of the sampled dolphins.

Table A4.29. Concentrations (ng/g lw) of α-HBCD in Atlantic white-sided dolphin

	Blubber			**Liver**		
Year	n	Min	Max	n	Min	Max
1993	3	164	360	3	5.8	36
1994	1	110	110			
1997	4	140	260	2	29	68
1998	19	19	350	6	3	13
1999	14	32	200	4	24	140
2000	6	76	330	1	26	26
2001	3	34	82			
2002	3	41	55			
2003	3	65	70			
2004	1	220	220			

No increasing or decreasing trends in HBCD concentrations in white-sided dolphin blubber were observed from 1993 to 2004.

Terrestrial invertebrates

No data are available.

Terrestrial birds and mammals

1. Muir et al. (2006) determined PBDEs in adipose tissue of adult and sub-adult female polar bears sampled between 1999 and 2002 from sub-populations in Arctic Canada, eastern Greenland and Svalbard, and in males and females collected from 1994 to 2002 in north-western Alaska. In bears from some of these locations, levels of HBCD were also determined. Subcutaneous adipose tissue samples were collected from harvested bears as part of the Inuit subsistence hunt in the Canadian Arctic, Alaska and Greenland, while, in Svalbard, fat biopsy samples were collected from free-ranging bears tranquillised for research purposes.

HBCD was present in 100% of the East Greenland samples, 100% of the Svalbard samples, and 13% of samples from the Bering–Chukchi. Analysis was done by GC, meaning that results are provided as total HBCD, due to the most likely thermal isomerisation of β- and γ-HBCD to alpha-HBCD through this process.

Table A4.30. HBCD concentrations (ng/g lw) in polar bear fat

Location	No. samples	Sex	Min	Max	Mean
Svalbard area	15	F	18.2	109	44.4
East Greenland	11	F	32.4	58.6	44.5
Bering–Chukchi Sea	8	F	<0.01	35.1	0.40
Bering–Chukchi Sea	7	M	–	–	<0.01

2. Lindberg et al. (2004) describe a study where several BFRs were analysed in peregrine falcon eggs. Eggs were collected within the Swedish Society for Nature Conservation inventory program. Egg contents were removed. Samples were from unfertilised eggs collected during the incubation period, or unhatched eggs collected after normal incubation was completed. Samples came from both a wild breeding population and a captive breeding population. For south-western Sweden (main diet for wild falcons being birds in the terrestrial food web), 24 eggs from 17 females were collected between 1992 and 1999 and 9 of these were analysed for HBCD. For northern Sweden (main diet for wild falcons being waders and ducks), 18 eggs from 18 females were collected between 1991 and 1999, and 8 were analysed for HBCD. For the captive breeding population (main food being a controlled diet of domestic chickens), 10 eggs from 8 females were collected between 1987 and 1999, and 4 were analysed for HBCD.

Table A4.31. Mean, range and geometric concentrations (ng/g lw) for HBCD in eggs from 3 different peregrine falcon populations

Population	No. Eggs	Mean	Range	Geometric mean
Captive	4	ND	<4–<8	ND
S. Sweden	9	520	79–2400	250
N. Sweden	8	220	34–590	150

Analysis was performed using GC/MS-ECNI. The method for HBCD is described as semi-quantitative. Due to likely thermal isomerisation through this process, HBCD levels are total levels only. The wild falcons had statistically significantly higher concentrations than the captive falcons.

These data were complemented by including an additional 31 eggs not previously analysed for HBCD (Johansson et al., 2009).

Table A4.32. Mean (range) concentrations (ng/g lw) of HBCD in eggs from 3 populations of peregrine falcons from Sweden

	Captive	Southern Sweden	Northern Sweden
HBCD	ND	270 (<10–2400)	210 (<9–1100)

HBCD was detected in all samples not included in the previous study. Although the HBCD method used in this study determines the total HBCD concentration, previous enantiomeric analysis of some eggs included in this study showed that only α-HBCD was present in the peregrine falcon eggs.

3. Murvoll et al. (2006) investigated the effects of various contaminants on retinoid and tocopherol status in European shag hatchlings (*Phalacrocorax aristotelis*). While the main outcomes of this study are considered in the Environmental Effects chapter of the main report, levels of HBCD found in the birds are reported here. Samples of plasma, liver and yolk sac were taken from 30 newly-hatched chicks. They were collected from 30 different nests at Sklinna, an island around 50 km off the coast of Mid-Norway. The chicks hatched from 8 to 14 June 2002. HBCD concentrations were determined by GC/MS. Mean values of HBCD found in the yolk sac were 28.5 (±18.8) ng/g ww, or 417 (±208) ng/g lw. These levels were around 1.6 times higher than total PBDEs (tri- to hexa-BDEs), but were around 40 times lower than total PCBs found in the same yolk sacs.

4. Leslie et al. (2004) report results of a study with the objective to determine concentrations of the 3 diastereomers of HBCD (α, β and γ) in eggs of the peregrine falcon (*Falco peregrinus*) and muscle tissue of sparrowhawks (*Accipiter nisus*) with liquid chromatography/mass spectrometry (LC/MS). *F. peregrinus* egg samples and *A. nisus* muscle tissue samples were provided by the specimen tissue bank of the Centre for Ecology and Hydrology (CEH) located at Monks Wood, Huntingdon, in the UK. All samples were collected in the UK, the *F. peregrinus* eggs between 1973 and 2002 and the *A. nisus* between 1975 and 2001. Two of the 51 *F. peregrinus* eggs and 4 of the 65 *A. nisus* muscle tissue samples were measured by both RIVO (Netherlands) and CEFAS (UK) laboratories. In the majority of samples tested (76% of *F. peregrinus* and 86% of *A. nisus* samples), HBCD diastereomers could not be detected. HBCD diastereomers were detected in 12 of the 51 samples of *F. peregrinus* eggs (range: 71 to 1200 ng total HBCD/g lw) and 9 of the 65 samples of *A. nisus* muscle tissue (range: 84 to 19,000 ng total HBCD/g lw). The highest HBCD concentrations were found in an *A. nisus* sample taken at an unknown location in 1995 and were considered more of an outlier than a sample from a peak year.

All 3 diastereomers were detected in both species, although the patterns varied from sample to sample. In *F. peregrinus* eggs, α-HBCD was found in all but 2 of the 12 samples in which HBCD diastereomers were detected. In those 2 samples, β-HBCD was the only diastereomer detected. No α-HBCD was detected in 4 of the 9 *A. nisus* samples. In these 4 samples, either β-HBCD or β- and γ-HBCD were detected. Six individual samples were analysed for HBCD by both RIVO and CEFAS: 2 *F. peregrinus* eggs and 4 samples of *A. nisus* muscle tissue. In one *F. peregrinus* egg, neither laboratory detected HBCD; in the second, both laboratories detected the α-diastereomer at

comparable levels of 20–27 ng/g ww (400–640 ng/g lw). In 3 of the *A. nisus* samples analysed by both laboratories, neither detected any HBCD. In the fourth sample, RIVO was able to detect 2.6 ng/g ww (84 ng/g lw), while the detection limit for this sample measured by CEFAS was <5.8 ng/g ww. The HBCD concentrations measured in this study do not suggest a trend of increasing or decreasing residues with sampling year, nor does it indicate particular peak periods in HBCD occurrence.

5. Sørensen et al. (2004) provide results of a project undertaken to study the contamination by xenobiotics and shell thickness of peregrine falcon eggs from Southern Greenland. Time trends and possible correlation profiles were investigated. Peregrine falcon (*Falco peregrinus*) eggs were collected in South Greenland between 1981 and 2003. Egg samples were taken from 28 different clutches and included 41 single eggs. The egg tissue concentration level was identified for a broad suite of 55 single chemicals including HBCD. The eggshell thickness was measured for the same eggs as used in the chemical analysis and also for shell fractions collected in other 47 clutches during the period of investigation. Total HBCD was detected in over half of the egg samples analysed, whereas the individual HBCD-isomers could not be detected due to higher limits of detection. The HBCD concentrations range from <0.1 ng/g lw to 230 ng/g lw in a sample from 1990. The median concentration was 9.5 ng/g lw, and the mean concentration was 28 ng/g lw. The HBCD concentrations measured in this study do not suggest a trend of increasing or decreasing residues with sampling year. These results are also reported in Vorkamp et al. (2005), although this latter publication states lower median and mean concentrations of 2.4 and 17 ng/g lw respectively.

6. In a time trend analysis of HBCD levels in guillemot eggs, Kierkegaard et al. (1999) report a significant increase over the period 1968 to 1997 from samples collected as part of the Swedish National Environmental Monitoring Program. While actual values are not reported, they are presented graphically. The general trend is upwards, with around 50 ng/g lw found in the eggs from 1968 compared to around 100 ng/g lw in the eggs from 1992. These levels, however, are based on a regression equation. Some significantly higher concentrations are reported. For example, eggs from 1976 and 1993 appear to have mean levels of around 150 ng/g lw and eggs from 1995 appear to have mean levels of around 170 ng/g lw.

7. An extension of this analysis is presented in Sellström et al. (2003), where trends in egg levels from 1992 to 2001 are considered. The arithmetic mean of 10 eggs per year from 1992–2001 were reported and showed mean HBCD concentrations ranging from 97 ng/g lw in 1992 (range of 63–120 ng/g lw) to 170 ng/g lw in 1995 (range of 100–300 ng/g lw). Mean levels in 2000 and 2001 were both 140 ng/g lw (range of 80–180 ng/g lw in 2000 and 64–220 ng/g lw in 2001). Statistical analysis of the full data (mid-1960s onwards) indicates a steady and significant (p <0.001) increase in concentrations over time up to the 1992 sampling, but this increase seems to have levelled out since the mid-1990s.

8. Lundstedt-Enkel et al. (2005) undertook a multivariate data analysis of various contaminants including HBCD and biological characteristics in adult Guillemot (*Uria aalge*) from the Baltic Sea. Ten males and 10 females drowned in trawl nets near Stor Karlsö were collected in 2000. For the chemical analysis, 10 g per individual of the right and left pectoral muscles were sampled and the lipid content of the muscles determined. In females, the geometric mean concentration of HBCD was 66.7 ng/g lw (95% CI 55.0–80.9 ng/g lw) while for males, the geometric mean HBCD concentration was 62.7 ng/g lw (95% CI 42.6–92.4 ng/g lw).

9. HBCD levels were examined in egg yolk and plasma of male and female glaucous gulls (*Larus hyperboreus*) from the Norwegian Arctic (Verreault et al., 2007). Samples of blood (plasma) were obtained in May and June of 2006, which corresponds to the nesting period in glaucous gulls, from incubating male (n = 19) and female (n = 30) glaucous gulls at Bear Island. In addition, unincubated, third-laid glaucous gull eggs (n = 31) from modal clutches of 3 eggs were collected randomly from the same colonial sites.

In male birds, 63% of plasma samples contained HBCD greater than the limit of quantification (LOQ = 0.63 ng/g ww) with a mean of 1.73 ng/g ww (range <0.63–5.73 ng/g ww). In females, 60% of plasma samples contained HBCD greater than the LOQ with a mean level of 2.07 ng/g ww (range <0.63–6.12 ng/g ww). All egg samples had quantifiable levels of HBCD with a mean of 19.8 ng/g ww and a range of 7.23–63.9 ng/g ww.

10. Helgason et al. (2009) assessed temporal trends (1983–2003) of HBCD in eggs of herring gulls (*Larus argentatus*), Atlantic puffins (*Fratercula arctica*), and black-legged kittiwakes (*Rissa tridactyla*) in North Norway. Eggs were collected in 2003 and samples also were taken retrospectively from eggs collected in 1983 and 1993. For all birds, n = 10 except for puffins in 1993, for which n = 9. After sampling, the individual eggs were homogenized, kept in the dark, and frozen until analysis. All eggs were analyzed in 2005–06.

Detectable concentrations of the α-HBCD isomer were present in all samples of eggs from the 3 species studied, whereas concentrations of the β-HBCD and γ-HBCD isomers were below the method detection limits in all samples. Levels of α-HBCD were highest in the black-legged kittiwake eggs and decreased in the order herring gull eggs then Atlantic puffin eggs. In 1983, 1993 and 2003, mean α-HBCD concentrations in black-legged kittiwake eggs were 30, 57 and 142 ng/g lw, in herring gulls were 16, 31 and 108 ng/g lw respectively and, in Atlantic puffin, were 12, 32 and 58 ng/g lw respectively. These results show increasing trends in the 3 bird species considered from 1983 to 2003.

A4.1.5 Monitoring data

Australian data

No Australian indoor or outdoor monitoring data are available for HBCD.

International data

Air

Outdoor Air

1. BFRs were analysed in archived air samples collected over 4 week periods in 1994-95 from Alert and Tagish in the Canadian Arctic and Dunai in the Russian Arctic (Alaee et al., 2003). Four samples each from Alert, Tagish and Dunai were analysed, with no HBCD detected in any of the samples (<1.8 pg/m^3).

2. Remberger et al. (2004) report on the environmental concentrations of HBCD in Sweden. Measurements were performed close to certain possible point sources in the urban environment and in remote regions and included air, deposition, water, soil, sediments, sludge, biota and foodstuffs. HBCD was detected in all media analysed, and

in all environments. Levels found are reported in the relevant subsections of this report. For air, HBCD attached to atmospheric particles was trapped on a glass fibre filter and HBCD in the gas phase was collected using adsorbent of polyurethane foam (PUF). At the background stations, a high volume air sampler (HVS) was used with a sampling flow rate of about 20 m^3/h. A low volume air sampler (LVS) was used for sampling at point sources with a flow rate of about 1 m^3/h. Deposition was collected using a bulk sampler consisting of a 1 m^2 teflon coated surface with 10 cm edges, equipped with a PUF containing cassette. The deposition sample included both wet and dry (that is, bulk) deposition. The atmospheric concentration at 2 potential point sources (a landfill site and a textile industry) showed HBCD concentrations were in the range 0.013–0.74 ng/m^3 (13–740 pg/m^3), while a single sample collected close to a factory producing XPS plastics returned a concentration of 1070 ng/m^3 (1 070 000 pg/m^3). At urban sites in Stockholm, concentrations of HBCD were 76 and 610 pg/m^3, while at more remote locations the range was <1–280 pg/m^3. These data suggest that HBCD may be sufficiently long-lived to undergo long-range atmospheric transport away from point sources of production and use (Law et al., 2006c). The atmospheric concentration of HBCD in background air was lower during the winter than the summer. The measured deposition of HBCD in the urban environment was nearly 70 times higher during the first sampling occasion than during the second occasion, although the precipitation was similar for the 2 events. The measured depositions at the background sites were lower in winter than summer. The deposition at the more remote site (Pallas) was about 10 times higher than that on the West Coast.

3. Hoh and Hites (2005) analysed HBCD and other BFRs in air samples from the East-Central United States. Air samples were collected at 5 sites (urban, semi-urban, agricultural and remote) from Lake Michigan through the US Midwest to the Gulf of Mexico every 12 d during 2002–03 using high-volume samplers so that the spatial trends could be investigated. HBCD was only detected in the particle phase. While the highest individual concentration found was in Arkansas (11 pg/m^3), based on mean concentrations, the highest level was in Chicago (mean, 4.5 pg/m^3 with a range of 0.9–9.6 pg/m^3, detected in 100% of the 28 samples). By contrast, the 2 remote sites (Michigan and Louisiana) had mean levels of 1.2 and 0.6 pg/m^3 respectively with HBCD found in 83% (29 of 35 samples) and 38% (10 of 26 samples) respectively. Interestingly, the highest concentrations found at these sites (8.0 and 6.2 pg/m^3 respectively) were not greatly lower than the highest level found in the Chicago monitoring. The relative abundance of the isomers for 7 samples was determined by LC/MS/MS. The results were variable. Three of the 7 samples were dominated by γ-HBCD, while one had approximately equal amounts of α- and γ-HBCD. Three samples were dominated by α-HBCD. The amount of β-HBCD (6%–17%) was low and not as variable as the other isomers.

4. Air samples were collected from 4 sites in Guangzhou, a typical fast-developing city of South China, for quantifying the concentration levels, diasteroisomer profiles, gas-particle distribution and enantiomeric fractions of HBCDs (Yu et al., 2008). Four sampling sites were chosen to encompass a range of aerosol types and potential sources. The following results were reported.

Table A4.33. Particle and gas phase concentrations (pg/m^3) of HBCD in Guangzhou

	Site 1	Site 2	Site 3	Site 4
	Mean particle phase concentrations			
α-HBCD	2.01	0.92	0.36	0.54
β-HBCD	0.33	0.18	0.08	0.10
γ-HBCD	0.64	0.42	0.16	0.15
	Mean gas phase concentrations			
α-HBCD	0.06	0.07	0.04	0.05
β-HBCD	0.03	0.03	0.02	0.02
γ-HBCD	0.02	0.02	0.02	0.02

The results showed that the measured mean atmospheric HBCD concentrations ranged from 0.69 to 3.09 pg/m^3. The analysis on diastereoisomer profiles indicated that α-HBCD (59%–68%) was the dominant isomer and β-HBCD was a minor isomer in all air samples, which appeared to be different from commercial products. A large but variable percentage of HBCDs (69.1%–97.3%) existed in the particle phase.

Sewage sludge

5. As part of their wider study, de Boer et al. (2002) undertook a sewage sludge and landfill study. STP and landfill samples were taken in The Netherlands, UK and Ireland.

Netherlands: At 5 STPs in The Netherlands, 4 with a high treatment capacity (200 000–750 000 population equivalents (pe)) (STP 1–4) and one small STP (100 000 pe) (STP 5), sewage sludge was sampled together with influent and effluent. At 4 other STPs, 2 small locations (150 000 pe) (STP 6 and 8) and 2 with a high treatment capacity (STP 7 (750 000 pe) and STP 10 (400,000 pe)), only sewage sludge was sampled. Besides these STPs, the sludge of the sewer from a residential area was sampled (STP 9).

The highest HBCD concentrations were found in the samples of the STP 2: 570 µg/kg dw in the influent residue, 93 µg/kg dw in the sludge and 140 µg/kg in the effluent. The other 4 influent samples, as well as the other 4 effluent samples, apart from that of STP 5 (100 µg/kg dw), showed non-detects for HBCD, but the sludge samples showed detectable HBCD levels. The samples from STP 1 were the only ones where HBCD was not found in any of the compartments. LC/MS analysis was performed to address isomer distribution. These varied between plants. Where influent and effluent levels were measured (5 STPs), α-HBCD accounted for 100% HBCD in the influent of 3 plants, and the effluent of 1 plant. Otherwise, in effluent, γ-HBCD seemed to dominate (up to 100%). In the sludge itself, the γ-HBCD isomer tended to dominate, but in many cases, significant levels (up to 50%) were found as α-HBCD.

United Kingdom: Sewage sludge, influent and effluent samples were taken from 5 locations in Essex: Burnham, Latchingdon, Wickford, S. Woodham Ferrers and Chelmsford, varying in population from 4750 (Latchingdon) to 143 000 (Chelmsford).

No HBCD was detected in the effluent of any of the plants in either the dissolved or particulate phase. In influent dissolved phase samples, HBCD was found in 4 of the 5 samples at 4.3–23.6 µg/kg dw. At the lower levels (4.3–9.1 µg/kg dw), all was attributed to γ-HBCD. The highest levels found (23.6 µg/kg dw) consisted of around 50% β-

HBCD, with around 33.5% and 13.5% α- and γ-HBCD respectively. In sludge, HBCD was detected at all STPs, with a range of 531–2683 µg/kg dw (mean 1401 µg/kg dw). Average residues consisted of 17%, 33% and 49.5% α-, β- and γ-HBCD respectively.

Ireland: In Ireland 3 STPs were sampled for sewage sludge: Portlaoise, Clonmel and Cork. Both the dissolved and the particulate phase of the influent and effluent samples were analysed. The particulate phase was obtained after filtration, over 0.45 µm PVDF filters. HBCD was found in the sludge at all plants. The isomer distribution varied between these sites.

Concentrations of HBCD detected in STP influent and effluent

	HBCD (µg/kg dw)	α-HBCD (%)	β-HBCD (%)	γ-HBCD (%)
Portlaoise	1439	23	30	47
Clonmel	210.5	7	–	93
Cork	8315	31	25	44

Values are means of 2 samples at each plant.

6. As part of a wider study, Remberger et al. (2004) sampled primary sewage sludge from a municipal STP near a laundry north of Stockholm. Further samples of primary and digested sewage sludge from the 3 larger STPs in Stockholm were also taken. While HBCD in digested sludge was below the 1 µg/kg dw detection limit, it was found in primary sludge with the highest value found in that of the STP near the public laundry.

7. A wider Swedish EPA study where sludge samples were collected in 2000 from 50 STPs and analysed for HBCD and other BFRs is reported in Law et al. (2006c). Here, HBCD was reported to be found in a range of 3.8–650 µg/kg dw with 2 to 8 times higher concentrations being found in sludge samples from a few STPs with known, or suspected, point sources connected to them. Otherwise, concentrations are stated to not vary much between STPs.

Sediment

8. Sellström et al. (1998) report levels of HBCD in sediment from a Swedish river. Surficial sediments (0–2 cm) were collected at 8 sites in the river Viskan and its tributary, the River Häggän, upstream and downstream from a number of possible point sources for BFRs as well as at one location in a nearby water system, with sampling performed in 1995. Recovery experiments described in the paper indicate the analytical method for determining HBCD was acceptable (recovery of 110%–124% in relation to the internal standard with no difference due to the spiking level). Of the sites sampled, HBCD was not detected at 2 sites (both upstream). At another 3 sites, based on 2 samples at each site, HBCD was not detected in one of the samples at each site, and found at levels up to 11 µg/kg IG (ignition loss basis) in the other sample. All these sites were in the vicinity of textile industries. Another site in the vicinity of a textile industry and an STP had mean levels of 230 µg/kg IG. The last 3 sites, further downstream with several textile industries and an STP upstream, had elevated levels of HBCD, ranging from 2700 to 7600 µg/kg IG.

9. A BSEF study on the occurrence of HBCD in the environment (sediments, biota and sewage sludge) has been performed (de Boer et al., 2002). The objectives of the study with respect to HBCD included analysing concentrations in:

- eel and sediments from the Scheldt Basin (Belgium)
- sewage sludges and landfill leachates from The Netherlands, the UK and Ireland
- sediments and biota from The Netherlands, the UK and Ireland, including the North Sea.

To avoid potential thermal rearrangements and co-elution during analysis with GC, LC/MS method was developed, which also included isomer specific determination of HBCD. The sediment findings are discussed below.

It is unclear how sediment sampling was performed, including the depth of sampling.

Scheldt Basin: In 2001, 19 samples from the Scheldt Basin were analysed. HBCD was detected in 12 of these, with levels ranging from 1.0–260 µg/kg dw. On an organic carbon basis, where detected, this range was 28–7200 µg/kg. It is reported that the γ isomer dominated in sediments (~90%). In the 2 sediments where the highest total HBCD levels were detected, elevated levels of α-HBCD were found (~20% total). This pattern is indicative for the presence of textile or plastic industry in the sampling vicinity.

Ireland: Four sediment samples analysed from Ireland showed detectable HBCD levels in 3 samples, with a range of 13.3–29.7 µg/kg dw. The γ isomer dominated (~100% in all 3).

In addition, 7 samples from Dublin Bay (2001) were analysed. The difficulty of these samples was the low TOC content. The samples were relatively sandy, making it difficult to measure detectable amounts of the target analytes. Using GC/MS, HBCD was found in 4 samples and ranged from 2.2–10 µg/kg (assumed to be dw). Where LC/MS was used to determine separate isomers, detectable levels were found in 3 samples, with γ-HBCD accounting for 100% in 2, and 92% in the third sample.

United Kingdom: Ten sediment samples from Tees in the UK found HBCD in 8 samples with a range of 11.8–511 µg/kg dw with a mean of 240 µg/kg dw where detected. γ-HBCD dominated the samples (mean 77% total HBCD levels where found) with a range of 58%–100% total levels. Levels of α-HBCD averaged 15% (0–21%) total while β-HBCD averaged 8% (0–22%) total levels. Two samples from 2000 in the River Tyne showed total HBCD levels of 14.2–321.7 µg/kg dw. The sample with the lowest levels was 100% γ-HBCD, while the sample with the highest levels contained 10%, 5% and 85% α-, β- and γ-HBCD respectively. Two samples from the River Skerne had 173.8 and 1678 µg/kg dw total HBCD. The sample with the lowest levels contained 44%, 24% and 32% α-, β- and γ-HBCD respectively, while the sample with the highest levels contained 23%, 9% and 68% α-, β- and γ-HBCD respectively. One sample from the River Humber showed 6.0 µg/kg dw HBCD, all present as γ-HBCD. HBCD was detected in 2 of 5 samples (from 2002) in the River Mersey. Both detections (22.3 and 52.1 µg/kg dw) consisted entirely of β-HBCD. Two samples from the River Clyde were taken in 2000, both with detectable levels of HBCD. One sample (7.0 µg/kg dw) was all present as γ-HBCD. The other sample (187.4 µg/kg dw) consisted of 34%, 7% and 49% α-, β- and γ-HBCD respectively.

Western Scheldt: From 19 samples taken in 2000, HBCD was detected in 12 samples and total HBCD levels ranged (where detected) from 1.1–128 µg/kg dw (99–8100 µg/kg

TOC basis). All sediment samples show a dominant presence of γ-HBCD (>90%). α- and β-HBCD were only occasionally found in relatively low levels.

Dutch rivers: In samples from 9 Dutch rivers in 2000, HBCD was detected in 7 of them with a range (where found) of 2.3–34 μg/kg dw (48–580 μg/kg TOC basis). The values found suggest that the HBCD found originated from Germany or further upstream in the River Rhine. Where characterisation of the HBCD isomers was performed, α-HBCD was detected in 2 samples, and made up 100% HBCD in one of these. β-HBCD was only found in one sample and accounted for 48% total HBCD in this sample. γ-HBCD ranged from 34%–100% total levels.

10. As part of a wider study on the environmental occurrence of HBCD in Sweden, Remberger et al. (2004) sampled sediments from various areas. Sediment from the sedimentation basin for leachate from a landfill site for construction and demolition waste (around 30 km north of Stockholm) was assessed. In addition, 6 surface sediment samples were taken along the river Viskan. These samples were all upstream of an STP although 4 were downstream from a textile industry facility. Also, deeper sediment samples (20–30 cm) from 3 stations representative of the urban environment were sampled. The levels varied from <0.1 to 25 μg/kg dw. In the textile industry area, concentrations were considerably higher downstream than upstream. No HBCD was found in the sedimentation basin at the landfill despite being found in the landfill leachate (3 ng/L). The measured concentrations in the urban sediments were all within the same range and were lower than those detected downstream of the textile industry. Levels were higher in the surface than in the deeper sediments.

11. Fish and sediments from 4 places along the Spanish River Cinca were analysed for HBCD (Eljarrat et al., 2004). The samples were collected upstream and downstream from Monzon, a heavily industrialized town draining into the river. HBCD was found in sediments at levels ranging from not detected to 514 μg/kg dw.

12. Klamer et al. (2005) report results of a study set up as an integrated survey of surface sediment designed to determine concentrations of selected substances in sediment extracts and the responses of a suite of bacterial bioassays to these extracts. Sampling sites were chosen so that different parts of the Dutch sector of the North Sea continental shelf were covered such as river outflows, coastal and remote areas. In the spring of 2000, surface sediments were sampled using a box-core; at each location the top 5 cm of 6 individual cores were pooled and further treated as one sample. Ten locations were sampled. For all analyses, the <63 μm grain size fraction was used. Total HBCD was determined and quantified at 9 of the test sites. Where quantified, levels ranged from 0.76 to 6.9 μg/kg (assumed dw). The mean level found was 3.8 μg/kg for the 9 sites.

13. Marvin et al. (2006) report the levels of HBCD in Detroit River suspended sediments. Suspended sediments were sampled monthly in the Detroit River in 2001 using single point sediment trap moorings from May to November at 9 stations ranging from the mouth at the outflow to western Lake Erie to the head in southern Lake St Clair. Each trap mooring consisted of 6 individual 1 m length sections of core tubing (7 cm internal diameter with the top of the tubes being around 1.5 m from the river bottom. Accumulated material deposited in the traps was removed monthly for analysis where each sample was homogenized. TOC levels typically ranged from 1%–2% and none of the stations exhibited any statistically significant seasonal variation in TOC. HBCD was analysed by LC with tandem MS (LC/MS/MS) allowing separation of the individual isomers. Concentrations ranged from <0.025–1.9 μg/kg dw (α-HBCD), <0.025–0.28

µg/kg dw (β-HBCD) and <0.025–2.3 µg/kg dw (γ-HBCD). Concentrations of total HBCD ranged from <0.075–3.7 µg/kg dw. Roughly two-thirds of HBCD profiles in suspended sediments were dominated by the γ isomer and were similar to profiles of commercial technical mixtures. Profiles of the remaining samples were dominated by the α-HBCD isomer with the β isomer consistently detected at substantially lower levels than the other 2. Seasonal sampling showed significant shifts in the relative ratios of the isomers. The spatial distribution of HBCD in the Detroit River was similar to other POPs (PCBs) and showed a strong association with urban/industrial activities in the watershed. However, the highest HBCD concentrations (2.6-3.7 µg/kg dw) were associated with areas of contemporary industrial activity and were much lower than maximum concentrations of PCBs (2200 µg/kg dw) found in areas of the Detroit River associated with historical industrial activity.

14. The chronology of HBCDs was investigated in 3 dated sediment cores from a prealpine lake (Lake Thun) in Switzerland (Bogdal et al., 2008). The sediment cores were sampled from the lake in 2005 and were divided into 1 cm sections. ΣHBCD appeared in SED1 in 1986, increased until 1993, remained constant from 1993 to 2000 (peak levels of 20–22 ng/g TOC), and then noticeably decreased in the 3 upper layers. In SED2, ΣHBCD appeared in 1978 and concentrations steadily increased between 1999 and 2004 (maximum level in 2004 of 54 ng/g TOC). In SED3, concentrations increased in 1983 and remained lower than in the other cores (maximum concentration in 2004 of 9 ng/g TOC). The increase of the sediment burden of HBCDs in the 1980–1990s corresponds to its market release as a flame retardant. Within the past few years, in the sediment from the shallow depositional zone near the entrance of the lake (SED1) concentrations have decreased, while increasing concentrations were observed in the middle of the lake in the deep depositional zone (SED2). Concentrations remained constant in the Western area (SED3). The decreasing trend in SED1 could conceivably indicate decreasing emissions of HBCDs into the environment. The observation of a decrease of HBCDs in core SED1 only, could result from faster sedimentation of suspended particulate matter from the water column in SED1, as this core provides from a shallow depositional zone of Lake Thun. The lower sedimentation of particles in the deeper zones of SED2 and SED3 might lead to a delay of the turning point in the HBCD trend.

A4.2 Effects on other organisms in the laboratory and field

A4.2.1 Avian toxicity

In a recent study (Fernie et al., 2009), HBCD was implicated as being partially responsible for delayed egg laying and smaller eggs being laid, causing thinner eggshells and differential weight loss during embryonic development, and reduced fertility and reproductive success in captive American ketstrels (*Falco sparverius*). The study was designed to identify potential changes in avian reproductive success and egg quality associated with exposure to environmentally relevant concentrations of DE-71 (commercial pentabromodiphenyl ether formulation). However, birds were unintentionally also exposed to HBCD, the actual source of which is unclear. The mean total α-HBCD concentrations in kestrel eggs were 0.002 ng/g ww in the control group, 0.68 ng/g ww in the low DE-71 exposure group and 15.61 ng/g ww in the high DE-71 exposure group. These levels were considered as being significantly higher in the high-exposure eggs, but similar between control and low-exposure eggs. The report notes that eggshell thickness was highly and negatively associated with all of the measured PBDE and total-α-HBCD concentrations, as was delayed egg laying and poorer egg qualities. These conclusions need to be treated with caution as HBCD was not the focus of this study and conclusions are drawn essentially on a single HBCD concentration (noting the "low" dose was not statistically different to control levels), and HBCD was present along with a large number of other polybrominated diphenyl ether congeners, that were the original focus of the study.

A4.2.2 Aquatic toxicity

1) Toxicity to fish

i) Acute toxicity

Graves and Swigert (1997a) tested the acute toxicity to rainbow trout (*Oncorhynchus mykiss*) in a 96 h flow-through system. The test was based on OECD TG 203 and was performed to GLP. The test substance consisted of a composite of HBCD samples from 3 manufacturers. The composite contained 6.0%, 8.5 % and 79.1% α, β and γ isomers respectively, with a total HBCD content of 93.6%.

Nominal test concentrations were based on the results of a range finding study and selected to be 0, 1.5, 2.2, 3.2, 4.6 and 6.8 µg/L. Mean measured concentrations were determined analytically from samples of test water collected from each treatment and control group at the beginning of the test and at approximately 48 h intervals. The concentration of DMF in the solvent control and in the HBCD treatment groups was 0.1 mL/L.

Test fish had an average length of 55 mm (range of 50–61 mm based on 10 negative control fish) at the end of the test. The average wet weight of these fish at the end of the test was 2.4 g (range 1.6–3.6 g). Loading (total wet weight of fish/L test water passing through the chambers in 24 h) was determined to be 0.27 g fish/L/day. Delivery of the test substance was initiated approximately 6 d prior to introducing fish. The test chambers were 25 L aquaria filled with approximately 15 L filtered well water (17.8 cm deep). Water was maintained at around 12 °C and the diluter was set to allow each chamber to receive around 6 volume additions of test water every 24 h. A photoperiod of 16:8 h light:dark was maintained.

Dissolved oxygen and pH measurements were made from each replicate test chamber at test initiation and at 48 and 96 h. Hardness, alkalinity, specific conductance, acidity and TOC were measured in the dilution water at test initiation and termination. Observations of mortality and other clinical signs were made approximately 1, 24, 48, 72 and 96 h after test initiation. The no mortality concentration and NOEC were determined by visual interpretation of the mortality and clinical observation data.

Measured concentrations (mean of 2 replicates at day 0 and day 2 sampling points) were 0.75, 1.5, 2.3, 2.3 and 2.5 µg/L. The 96 h measured concentrations were artificially high due to co-eluting artefacts at the retention time of HBCD, and these could not be separated. Therefore, these measurements were not used in determining actual concentrations. Overall, it appears that the solubility limit of HBCD, under the test conditions, was within the range of 2.0–3.0 µg/L and the exposure concentrations were apparently at the solubility limit for HBCD in the 3 highest nominal exposure groups. This solubility limit is in good agreement with solubility results for HBCD, despite the use of DMF that could possibly have increased its water solubility.

Dissolved oxygen ranged from 9.6–9.9 mg/L at 0 h, 9.1–9.4 mg/L at 48 h and 8.4–9.2 mg/L at 96 h, with the lowest levels associated with the highest exposure concentrations. The pH remained at 8.2 to 8.3 throughout the test.

No mortality or sublethal effects were seen in any fish at any of the exposure concentrations throughout the test. Based on this test, HBCD appears to have no effect on rainbow trout up to its limit of water solubility.

ii) Chronic and sub-chronic toxicity

Drottar et al. (2001) evaluated the toxicity of HBCD during early life-stage development of rainbow trout (*Oncorhynchus mykiss*). Hatching success, time to hatch, time for larvae to swim up and post-hatch growth and survival were evaluated during the 88 d test. The study was based on OECD TG 210 and OPPTS 850.1400 and followed GLP.

The test substance was a composite of 3 samples and contained 9.4%, 6.3% and 84.3% α-, β- and γ-HBCD respectively. Total purity was 100%. Stock solutions were prepared by dissolving the test substance in acetone. Rainbow trout embryos were exposed to a geometric series of nominal 0.43, 0.85, 1.7, 3.4 and 6.8 µg/L, along with a control and solvent control under flow-through conditions. Four replicate test chambers were maintained in each group, with each containing 2 incubation cups. Each incubation cup contained a nominal count of 15 embryos (total of 30 per treatment and 120 per experimental group). An additional 30 embryos were held in dilution water and sacrificed on day 11 to evaluate fertilisation success. The 88 d test period included a 27 d hatching period and a 61 d post hatch period. Test concentrations were analytically determined at weekly intervals and at test termination.

Daily observations were made during embryo incubation and post-hatch exposure to evaluate sub-lethal effects. Post-hatch growth was measured on day 29 post-hatch and at the conclusion of the test. At test termination, total lengths for each surviving fish were made and wet/dry weights were measured.

The mean measured concentrations for the study were 0.25, 0.47, 0.83, 1.8 and 3.7 µg/L, representing 49%–58% of nominal concentrations. Dissolved oxygen concentrations remained at least 6.6 mg/L (61% saturation), and pH ranged from 7.6 to 8.1. The water hardness was 120–136 mg $CaCO_3$/L, with alkalinity of 179–184 mg $CaCO_3$/L in the negative control group. In the highest exposure group, these values were similar.

Fertilisation success as determined from embryos maintained in dilution water under test conditions at day 11 was 99.2%. There was no apparent difference in time to hatch between control groups and treatment groups. Embryos began hatching on day 23 and all surviving embryos in all groups had hatched by day 33. Hatching success in all HBCD groups was at least 83% compared with 75% and 85% in the control and solvent control respectively. No treatment-related effects were observed for hatching success.

Larvae began swimming up from the bottom of the test chambers on day 13 post-hatch, with 97% of control fish attaining swim-up by day 22 post-hatch compared with 96%-99% in the treatment groups. At this time all chambers were thinned to 15 fish. There was no statistically significant reduction in the numbers of fish swimming up in any treatment group compared to the pooled controls.

Survival was analysed for the post-hatch periods up to the thinning stage, and from then to test termination. Mean control survival prior to thinning was 97% compared to ≥97% in the treatment groups. After thinning, mean control survival was 98% compared to ≥97% in the treatment groups.

There were no statistically significant difference in the growth measurements (length and wet weight) between control groups and treatment groups.

All fish appeared normal and healthy throughout the study period. In this test, HBCD was not chronically toxic to rainbow trout in their early life stage up to the level of solubility. The NOEC was determined to be 3.7 µg/L.

2) Toxicity to aquatic invertebrates

i) Acute toxicity

The acute toxicity of HBCD to *Daphnia magna* was evaluated in a 48 h flow-through study performed according to OECD TG 202 and GLP (Graves & Swigert, 1997b). The test substance consisted of a composite of HBCD samples from 3 manufacturers. The composite contained 6.0%, 8.5 % and 79.1% α, β and γ isomers respectively, with a total HBCD content of 93.6%. There was no information on the identity and properties of the remaining 6.4%. A stock solution was prepared by dissolving HBCD in dimethylformamide (DMF).

Nominal test concentrations were 0, 1.5, 2.2, 3.2, 4.6 and 6.8 µg/L. Daphnid neonates (<24 h old) were used for the test. The control, solvent control and each test group consisted of 2 replicates, each containing 10 animals.

The pH and dissolved oxygen were measured at 24 h intervals. Observations were made to determine the number of mortalities/immobilisation and behaviour at 2, 24 and 48 h. The NOEC was determined by visual interpretation of the observations.

Exposure concentrations were steady, although lower than the nominal concentrations. Mean measured concentrations were 2.4, 1.8, 2.1, 2.3 and 3.2 µg/L. The authors suggest the unexpected pattern of measured HBCD concentrations may reflect a phenomenon in the delivery system whereby HBCD absorbed to the physical surfaces of the diluter system and the concentrations of HBCD in the dilution water were approximately the solubility of HBCD in the well water.

Water temperatures were within 2 °C of the target temperature and dissolved oxygen concentrations at least 97% of saturation throughout the test. Measurements of water pH ranged from 8.1 to 8.4. The hardness and alkalinity of the dilution water measured prior to the test initiation were 132 to 136 and 178 to 180 mg $CaCO_3$/L respectively.

No mortalities or other signs of toxicity were observed in the negative and solvent control groups throughout the test. The same was observed in all treatment groups with the exception of one mortality in the second highest treatment group. In this test, HBCD had no effect on daphnids up to its level of water solubility.

ii) Chronic toxicity

Drottar and Krueger (1998) evaluated the chronic toxicity of HBCD on survival, growth and reproduction of *Daphnia magna* during a 21 d exposure period under flow-through conditions. The test was performed according to OECD TG 202 and GLP. The test substance consisted of a composite of HBCD samples from 3 manufacturers. The composite contained 6.0%, 8.5 % and 79.1% α, β and γ isomers respectively with a total HBCD content of 93.6%. There was no information on the identity and properties of the remaining 6.4%. A stock solution was prepared by dissolving HBCD in dimethylformamide (DMF).

Nominal test concentrations were 0, 0.85, 1.70, 3.40, 6.80 and 13.6 µg/L. Two replicate test chambers were used in each group, with each treatment consisting of 4 test compartments containing 10 daphnids. Concentrations were analytically verified at test initiation, and at days 7, 14 and 21.

Young daphnids (<24 h old) were used in the test. Observations of each first-generation daphnid were made daily during the test for survival, the onset of reproduction and clinical signs of toxicity. With the onset of reproduction, the number of live and dead second-generation daphnids were counted regularly. At the end of the test, the length and dry weight of each live first-generation daphnid was determined.

Unlike the acute toxicity studies on fish (rainbow trout) and *Daphnia magna* following this same approach, in this case, measured concentrations were well characterised and ranged from 81% to 102% of nominal concentrations. Measured exposure concentrations were established to be 0.87, 1.6, 3.1, 5.6 and 11.0 µg/L, with relatively constant concentrations found in each treatment group over the course of the study.

Water temperatures ranged between 19.9 and 20.1 °C, dissolved oxygen gradually decreased from 8.7–8.8 mg/L at day 0 to 7.4–8.4 mg/L at day 21 (but was always at least 79% saturation) and pH was between 8.1 and 8.4 over the course of the study. Hardness and alkalinity in the negative control water ranged from 128 to 132 and 176 to 178 mg $CaCO_3$/L respectively.

The following table summarises the results.

Table A4.34. Summary of survival, reproduction and growth endpoints in 21-day *Daphnia* study

Endpoint	Exposure concentration (µg/L)						
	Negative control	**Solvent Control**	**0.87**	**1.6**	**3.1**	**5.6**	**11**
Day 21 mortality (%)	5	2.5	2.5	2.5	0	7.5	12.5
Mean young/ reproductive day	3.62	3.85	3.72	3.86	3.83	3.51	2.84*
% of pooled controls			99.6	103.3	102.5	94.0	76.0*
Mean length (F0, mm)	4.09	4.07	4.08	4.05	4.04	4.01*	3.87*
% pooled controls			100.0	99.3	99.0	98.3*	94.9*
Mean weight (F0, mg)	0.69	0.68	0.7	0.67	0.68	0.66	0.56*
% pooled controls			102.2	97.8	99.3	96.4	81.8*

* = Statistically significantly different from control ($p \leq 0.05$).

Statistical analysis of mortality showed that mortality in all treatment groups was not statistically different from the pooled controls. Surviving first-generation daphnids in all groups were normal with no unusual behaviour throughout the study, with the exception of 2 animals noted as being lethargic in the 3.1 ppb group at 48 h.

No statistically significant effects on survival, reproduction and growth were observed at 3.1 µg/L or less. Therefore, the 21 d NOEC was determined to be 3.1 µg/L, which is approximately the water solubility of HBCD. Based on effects on reproduction, the LOEC was determined to be 5.6 µg/L, with a resulting MATC of 4.2 µg/L.

3) Toxicity to algae / aquatic plants

Roberts and Swigert (1997) evaluated toxicity of HBCD to the freshwater green alga (*Selenastrum capricornutum*) in a 96 h exposure study. The test was based on OECD TG 201 and was performed to GLP. The test substance consisted of a composite of HBCD samples from 3 manufacturers. The composite contained 6.0%, 8.5 % and 79.1% α, β and γ isomers respectively with a total HBCD content of 93.6%. There was no information on the identity and properties of the remaining 6.4%. A stock solution was prepared by dissolving HBCD in dimethylformamide (DMF).

Nominal test concentrations were 1.5, 2.2, 3.2, 4.6 and 6.8 µg/L. In addition, abiotic (no algae) analytical test solutions were maintained for measuring test concentrations at the end of the study.

Test solutions were inoculated with 1.0 mL of an inoculum with approximate density of 1 x 10^6 cells/mL to achieve a final cell density of 1 x 10^4 cells/mL. Samples of the test solutions were collected from each replicate test chamber at 24 h intervals to determine cell densities. Cell densities and area under the growth curve (AUC) were determined for each replicate to calculate percent inhibition values relative to the controls.

The EC10, EC50 and EC90 values were calculated based upon cell densities and AUC values for each 24 h interval. The NOEC was determined based upon statistical evaluation of the cell densities and AUC values.

At test initiation, pH remained at 7.4–7.5 and after 96 h ranged from 8.0–8.4 in controls and test groups. The day 0 measured concentrations were in reasonable agreement with nominal concentrations, ranging from 87%–106% nominal respectively. However, the day 4 (96 h) measured concentrations from the abiotic controls did not agree well, ranging from not quantifiable to 59% of nominal. Measured concentrations at day 0 and day 4 are presented as follows.

Table A4.35. Nominal and measured concentrations (µg/L)

Nominal concentration	Measured day 0	% nominal	Measured day 4	% nominal
1.5	1.30	87	<0.57	NQ
2.2	2.25	102	1.20	55
3.2	3.38	106	1.90	59
4.6	4.28	93	1.64	36
6.8	6.44	95	<1.94	NQ

NQ = not quantifiable.

The following inhibition results were found (in terms of nominal concentrations).

Table A4.36. Percent inhibition values (cell densities) for each 24 h interval relative to pooled controls

Exposure concentration (µg/L)	24 h	48 h	72 h	96 h
1.5	-14	2.7	-0.25	-1.0
2.2	-16	-15	11	-8.5
3.2	-8.6	-19	-7.5	-2.2
4.6	-20	-14	2.0	-2.6
6.8	3.3	-4.2	16	7.8

Table A4.37. Percent inhibition values (AUC) for each 24 h interval relative to pooled controls

Exposure concentration (µg/L)	24 h	48 h	72 h	96 h
1.5	-15	-5.5	-0.93	-3.8
2.2	-17	-21	0.38	-5.4

3.2	-6.9	-21	-12	-9.2
4.6	-22	-20	-5.0	-5.4
6.8	9.1	-3.9	9.1	9.9

There was no noticeable change in cell colour, size or morphology in any treatment group when compared to the controls. No evidence of clumping, flocculation or adherence of the algae to the test flask was found during the visual and microscopic examination of algal cells from each treatment group.

No dose response was found. Inhibition of around 10% based on AUC after 96 h was observed in the highest tested treatment. However, the findings are inadequate to calculate EC50 results. Averaging the measured concentrations at the start and the end of the test for the highest test group results in a mean exposure concentration of 3.7 µg/L (corrected value based on recovery). This must be considered the NOEC for the test, and approximates the measured water solubility of total HBCD.

Desjardins et al. (2004) determined the toxicity of water-soluble components of HBCD to the marine diatom (*Skeletonema costatum*) using saltwater algal media passed through a generator column saturated with HBCD. The test was based on OECD TG 201, ISO 10253:1995 and EU Method C.3. and GLP. The test substance was a composite of 3 samples with a purity of 95.86%, and isomer distribution of 7.67%, 5.15% and 83.04% α-, β- and γ-HBCD respectively.

The marine diatom was exposed to a single test concentration of HBCD, a negative control and a media control (no generator column) for 72 h. Measured test concentrations (as separate α, β and γ isomers) were determined from samples of test medium collected from the treatment and each control group and the beginning and end of the test.

At test initiation, an inoculum of the algal cells was added to each test chamber at a concentration of 77 000 cells/mL. Samples were collected from each replicate test chamber at 24 h intervals to determine cell densities and AUC values.

The arithmetic mean of total HBCD at test termination was 41.0 µg/L, and consisted of mean measured test concentrations for α-, β- and γ-HBCD of 30.5, 8.86 and 1.61 µg/L respectively.

Inhibition was observed based on both cell density and AUC. Based on cell density, inhibition was 19% and 31% compared to the control with no generator column and with the generator column, respectively. Based on AUC, inhibition was 21% and 31% compared to the control with no generator column and with the generator column, respectively. Because only one concentration was tested, it is not possible to calculate a NOEC, or EC50. However, it can be stated that the NOEC is lower than the tested level – that is, <41 µg/L, while the EC50 is >41 µg/L.

Walsh et al. (1987) studied the toxicity of HBCD to the marine unicellular algae *Skeletonema costatum, Thalassiosira pseudonana* and *Chlorella* sp. The tests were carried out for either 72 h (*S. costatum* and *T. pseudonana*) or 96 h (*Chlorella* sp.). HBCD was obtained from Great Lakes Chemical Corp., but no further characteristics are given. The endpoint measured was the EC50 for growth based on population density, which was estimated by cell counts on a hemacytometer. Each test was replicated. HBCD was introduced into growth flasks by adding 0.05 mL in nanograde acetone to 51 mL growth medium with algae (1 mg/L). The highest concentration to be used in the test was determined by adding the substance slowly to growth medium and

observing the highest concentration at which crystals did not form. The estimated concentration for saturation was 1.5 mg/L. The concentration of HBCD in the stock solution and exposure media was confirmed by capillary column gas-liquid chromatography. Tests were performed in 2 replicates for each medium. The EC50 was derived by straight line graphical interpolation without calculation of confidence intervals.

Six different growth media were used in the test, one natural sea water and 5 synthetic sea water formulations (5 growth media tested with *S.costatum*). The natural sea water had a salinity of 32‰ and was diluted to give a final test salinity of 30‰ to be comparable with that of the synthetic media. The pHs of the various test media were in the range 7.6–8.2.

Chlorella sp. was not inhibited by as much as 50% at 1.5 mg HBCD/L. However, HBCD appeared toxic to *S. costatum* and *T. pseudonana* below the estimated saturation concentration. EC50 values were determined in 4 of the 5 test media for *S. costatum* and ranged from 9.0 to 12.2 µg/L. This species was more sensitive than *T. pseudonana*, where EC50 values were determined in all 6 test media and ranged from 50–370 µg/L. The lowest EC50 for this species approximates the maximum water solubility of HBCD in salt water as determined above in Desjardins et al., (2004). However, the results for *S. costatum* were all below the apparent maximum water solubility of HBCD. With multiple data available for the same species with the same endpoint, the geometric mean value can be used. In this case, the geometric mean EC50 for this species was determined to be 10.5 µg/L. For this species, there was little variability between the results (standard deviation = 1.05) regardless of the test medium.

4) Sediment-dwelling organisms

Thomas et al. (2003a) performed a prolonged sediment toxicity test with the amphipod *Hyalella azteca* using a flow-through test system with sediments of nominal 2% organic carbon content. The test protocol was based on the ASTM E 1706-95b Guideline and USEPA Series 850 Ecological Effects Test Guidelines (OPPTS No. 850.1735). The test substance consisted of a composite of HBCD samples from 3 manufacturers. The composite contained 7.67%, 5.15% and 83.04% α, β and γ isomers respectively with a total HBCD content of 95.86%. Artificial sediment was used for the experiment using α-cellulose as its source of organic matter. The dry sediment had a mean organic matter content of 3.9% (mean OC of 2.3%), WHC of 9.3%, pH of 8.1, and particle distribution of 84% sand, 3% silt and 13% clay.

Nominal test concentrations were prepared by weighing the test substance and adding directly to the dry sediment. This was then mixed for around 23 h. Nominal test concentrations were 31, 63, 125, 250, 500 and 1000 mg/kg dw sediment. Overlying water, pore water and sediment samples were collected and analysed from the analytical replicates of the control group and the lowest and highest test concentrations, on days 0 and 7 and at the end of the study.

Eight replicate test compartments were prepared for each treatment and control group. An additional 6 replicates were prepared for those treatments where analytical measurement of concentrations was performed. For each, 100 mL of sediment was placed in a 300 mL glass beaker and overlying water (~100 mL) added to the test compartments. The sediment/water mixtures were allowed to acclimate for around 2 d prior to introducing the test organisms. At test initiation, amphipods were added impartially until each container contained 10 animals. The dilution water used for culturing and testing was freshwater from a well characterised as moderately hard (hardness 120–140 mg/L as $CaCO_3$; alkalinity 182 mg/L as $CaCO_3$ and pH of 8.2 to 8.4). The water was filtered to remove microorganisms. Flow-through conditions were maintained during the exposure period. The depth of overlying water in each test compartment was maintained by the water levels in the diluter tanks and the diluter was adjusted so that each tank received around 2 volume additions of water per day. The exposure period was 28 d. The water depth in one representative test compartment was 6.8 cm, with the depth of sediment in one representative test compartment being 2.8 cm.

A photoperiod of 16:8 h light:dark was used and the target test temperature was 23±2 ^{o}C. The test chambers were observed at least 3 times per week to determine mortalities and sub-lethal effects. At test termination animals were segregated from the sediment, and the numbers of live or dead amphipods were counted. Surviving animals were rinsed of excess sediment and dry weight recorded.

Results were based on the day 0 nominal sediment concentrations. The NOEC and LOEC were determined by visual interpretation of the dose-response pattern and statistical analysis of the survival/reproduction and dry weight data. The data did not allow for determination of an EC50.

Sediment concentrations measured in the 31 mg/kg treatment group were variable and ranged from 49.5% to 125% in the 2 replicates at day 0, and between 53.2% and 94.3% at other sampling times. Based on 3 measurement days (0, 7 and 28) with a total of 6 samples, the mean measured concentration was 24.5 mg/kg (79% nominal). In the 1000 mg/kg group, levels were much more consistent in the sediment, ranging from 82.8 to 115% nominal, with a mean measured concentration over the course of the study of 1011 mg/kg (101% nominal). No HBCD was detected in overlying water samples at any time, although the LOQ (0.1 mg/L) was much higher than the water solubility of HBCD. In pore water, levels for the 31 mg/kg group were all below the mean LOQ of 0.46 mg/L (much higher than the water solubility level). However, concentrations measured in the 1000 mg/kg treatment group were reported at a mean level of 3.18 mg/L, significantly higher than the water solubility of the compound. They are probably the result of small particles of HBCD being extracted out of the pore water, artificially inflating the reported values. Temperature remained within the target range, and dissolved oxygen concentrations were >65% throughout the test. The pH ranged from 7.8 to 8.6 during the study.

The presence of fungal growth was noted in all replicates in all treatment groups and the control during the test. The following results were found.

Table A4.38. Mean survival and growth of amphipods – 28 d, 2% OC sediment toxicity test

	Nominal concentration (mg/kg dw sediment)						
	0	31	63	125	250	500	1000
Mean number surviving amphipods	7.4	5.9	7.8	5.4	6.9	7.3	5.8
Per cent reduction		20	-5.4	27	6.8	1.4	22
Mean individual dry weight (mg)	0.11	0.10	0.13	0.14	0.12	0.17	0.14
Per cent reduction		4.6	-22	-32	-8.3	-59	-29

The mean number of amphipods in the treatment groups was not statistically different (p >0.05) from the control group. The dry weights in the treatment groups were not significantly different from the control weights. The 28 d EC50 for amphipods exposed to HBCD in sediment with 2.3% OC was >1000 mg/kg dw sediment, the highest rate tested. Based on the results of this study, the LOEC was >1000 mg/kg dw sediment and the NOEC was 1000 mg/kg dw sediment.

A second study following the same methodology and test concentrations was performed using a 5% OC (actual, 4.7%) sediment (Thomas et al., 2003b). In this study, the following results were found.

Table A4.39. Mean survival and growth of amphipods – 28 d, 5% OC sediment toxicity test

	Nominal concentration (mg/kg dw sediment)						
	0	31	63	125	250	500	1000
Mean number surviving amphipods	9.1	8.6	5.9	6.1	7.0	8.5	9.1
Per cent reduction		5.5	35.2*	33.0*	23.1*	6.6	0
Mean individual dry weight (mg)	0.19	0.17	0.26	0.22	0.20	0.19	0.19
Per cent reduction		10.5	-36.8	-15.8	-5.3	0	0

* = statistically significantly different to control ($p \leq 0.05$).

All replicates observed during the test appeared normal, with mortality significantly different from control replicates in the 63, 125 and 250 mg/kg treatment groups. However, given the lack of mortality in the higher treatment groups, these findings are not considered to be treatment-related. No statistically significant effects on growth (dry weight) were found at any treatment level. The 28 d EC50 for amphipods exposed to HBCD in sediment with 4.7% OC was >1000 mg/kg dw sediment, the highest rate tested. Based on the results of this study, the LOEC was >1000 mg/kg dw sediment and the NOEC was 1000 mg/kg dw sediment.

The organisms were fed trout chow during the tests. A 1.0 mL aliquot of food was added to each test compartment roughly daily during the test. This food was not spiked with HBCD. The EU Technical Guidance Document (EC, 2003) indicates that for highly adsorptive substances, food could be an important exposure pathway in this type of sediment toxicity test, and recommends that the test method used should try to ensure

that exposure via this route cannot be avoided in the test. In this case, as HBCD was not present in food, it is possible that the results of the test could underestimate the actual toxicity, although the significance of this route of exposure for HBCD is unknown.

A4.2.3 Terrestrial toxicity

1) Plants

The toxicity of HBCD to 6 species of plants has been determined using OECD Guideline 208 (the protocol is based on the 1998 proposal for revision of this test guideline) and OPPTS 850.4100 and 850.4225 in a seedling emergence study (Porch et al., 2002). The test substance consisted of a composite of HBCD samples from 3 manufacturers.

An artificial soil was composed of kaolinite clay, industrial quartz sand and peat in a 4:50:2 w:w:w ratio. Crushed limestone was added to buffer the pH of the soil. The soil characteristics were 53%, 30% and 17% sand, silt and clay respectively with pH of 7.5 and 1.9% OM.

Nominal test concentrations were 0, 40, 105, 276, 725, 1,904 and 5000 mg/kg soil dw. The concentrations were verified analytically. Three monocots (corn, onion and rye grass) and 3 dicots (cucumber, soybean and tomato) were tested. For each species, a control group, and the 5 treatments were maintained. Each group consisted of 4 replicates each containing 10 seeds. During the 21 d test, weekly observations of seedling emergence and a qualitative assessment of the condition of each seedling were made. At the termination of the test the growth of the seedlings was evaluated in terms of mean shoot height and fresh weight.

Mean seedling emergence, survival, weight and height of the control and treatment groups were compared with Dunnett's t-test. Significance was determined at the level of 0.05.

The mean measured concentrations (mean of 3 samples from each treatment level) resulted in total HBCD concentrations of 31.2, 97.7, 297.1, 764.6, 2230 and 6200 mg/kg dw. These concentrations were dominated by γ-HBCD (mean 86.1%) with mean levels of α- and β-HBCD accounting for 7.9% and 6.0% respectively.

There were no apparent effects on any endpoint for any of the 6 species tested. Statistical analyses indicated no significant differences between the control and treatment group mean emergence, survival, height or weight for corn, cucumber, ryegrass, soybean and tomato. On day 21, onion showed significant differences between the control and the 276 mg/kg group mean survival. This was not considered treatment-related due to no effect on this endpoint at higher concentrations. Also, there were no statistical differences between the control and group mean emergence, height or weight for onion. There were no signs of treatment-related phytotoxicity observed on seedlings of any species at any test concentration.

Overall, the NOEC from these studies was ≥6200 mg total HBCD/kg dry soil based on the mean measured concentration in soil at the start of the test.

2) Earthworms

A reproduction test with earthworms (*Eisenia fetida*) was performed following OECD TG 207 and OPPTS 850.6200 (Aufderheide et al., 2003). The test substance was HBCD

with an isomer distribution of 5.79%, 19.32% and 74.89% α-, β- and γ-HBCD respectively. Artificial soil (80% sand, 8% silt and 12% clay) was used, with the pH adjusted to 6.5. The WHC was 56.8% and organic carbon of 4.3%. Test soils were prepared by adding the test substance in the form of pulverised HBCD to air-dried soil to give nominal test concentrations of 78.5, 157, 313, 625, 1250, 2500, and 5000 mg/kg dw soil. The water content was adjusted to 60% of WHC. Around 611 g of hydrated soil (500 g dry soil) was added to 4 replicate test chambers per treatment and 8 for the control group. At the start of the test, worms (10 per replicate) were placed on the surface of the soil and observed for burrowing behaviour. The chambers were maintained at around 20 °C during the test. A 16:8 h light:dark photoperiod was maintained. The test was performed for 56 d. During the first 28 d, worms were fed 3–4 mL of invertebrate diet slurry at least twice a week, with the food buried below the soil surface. During the final 28 d, 3 mL of the diet slurry was added just below the soil surface at least once a week. Additional water was added as required.

Adult worms were used for the test. The mean weight of the worms at start of the test was 433.2 mg/worm in the control population and 354.0–502.6 mg/worm in the treatment groups. After the first 28 d, adult worms were removed and mortality/movement recorded. They were purged of their gut contents and tissue concentrations analysed. After 56 d, juvenile animals were counted.

The concentration of HBCD present in the soil was measured at day 0, day 28 and day 56 of the test. The homogeneity of the samples was checked by measuring the concentration in samples from the top, middle and bottom of the soils after mixing/hydration of the highest and lowest exposure concentrations. Concentrations measured in the soil and worm tissue (28 d) were found as follows.

Table A4.40. HBCD concentrations in soil (mg/kg dw) and worm tissues (µg/g)

Nominal concentration	78.5	157	313	625	1250	2500	5000
Day 0 measured	73.7	165	268	592	1290	2460	4680
Mean day 28	61.2	145	244	578	1150	2180	4190
Mean day 56	51.5	128	235	543	1070	2020	3990
Day 28 tissue	3.4	7.32	16.8	15.3	53	71.2	150

In tissue samples, α-HBCD dominated the isomer profile, ranging from 51.8% to 73.1% of total HBCD found in tissues. γ-HBCD was next most abundant (13.8%–37.7% total HBCD) and β-HBCD was found at the lowest levels of 10.4%–17% total HBCD.

No abnormal burrowing or avoidance behaviour was seen in the first 60 minutes of the test.

Table A4.41. Effects of HBCD on earthworm mortality and weight (28 d) and reproduction (56 d)

Nominal concentration	0	78.5	157	313	625	1250	2500	5000
After 28 days								
Mortality (%)	0	5	0	0	0	0	3	0
% Weight gain	10	19	11	11	3	19	0.4	11
After 56 days								
Juveniles/replicate	72	61	60	49*	31*	26*	26*	30*

* = statistically significantly different to control ($p \leq 0.05$).

The 28 d mortality NOEC was 4190 mg total HBCD/kg dw soil. The estimated EC_{10} and EC_{50} values for adult earthworm survival were >4,190 mg total HBCD/kg dw soil. The 56 d reproduction NOEC was 128 mg total HBCD/kg dw soil. At the lowest rate tested, there was still a 15% reduction on reproduction. Consequently, the reproduction NOEC value was estimated using a one-way analysis of variance (ANOVA) procedure and a one-tailed Dunnett's test. The reproductive data were shown to satisfy the assumptions of normality and homogeneity of variance and the ANOVA was performed on the raw data. The estimated EC_{10} and EC_{50} values for average reproduction were 21.6 mg/kg dw soil (95% CI 0.000468–110 mg/kg dw soil) and 771 mg/kg dw soil (95% CI 225–4900 mg/kg dw soil) respectively.

While HBCD was found in earthworm tissues, it was not particularly bioaccumulative. Bioaccumulation factors (concentration in tissue/concentration in soil) were all within the range of 0.026–0.069.

A4.2.4 Endocrine disruption effects

FIRE project

A multidisciplinary project, "Risk Assessment of Brominated Flame Retardants as Suspected Endocrine Disruptors for Human and Wildlife Health" also known as "FIRE project" (Flame retardants Integrated Risk assessment for Endocrine disruption) (FIRE) was undertaken in Europe from 2002 to 2006. The main objective of the project was an integrated approach of directed toxicological studies and exposure assessments to characterise the possible emerging health risk for humans and wildlife of these compounds by endocrine-related mechanisms (http://www.rivm.nl/fire/).

BFRs, including PBDEs, tetrabromobisphenol A (TBBPA) and HBCD were identified as potential endocrine disrupters and were subjected to in-vitro screening to determine any possible endocrine-related mechanisms. A battery of in-vitro assays (human, rat and fish cell lines) and QSAR models were used. The final report summary of the project has now been released by the EU (http://ec.europa.eu/research/endocrine/pdf/fire_finalreport_summary.pdf).

The project consisted of 7 themes, as follows:

Theme 1:Chemical synthesis and prescreening of BFRs

Themes 2 and 3: Human and wildlife hazard identification and dose-response assessment

Themes 4 and 5: Human and aquatic wildlife exposure assessment

Theme 6: Integrated risk assessment for humans and aquatic wildlife

Theme 7: Coordination, dissemination and cluster activities

Under Theme 1, BFRs were selected for in-vitro screening activity to determine any possible endocrine-related mechanisms. The total test set of 27 different compounds consisted of 19 different PBDEs, the technical mixture of hexabromocyclododecyl (HBCD) and its 3 individual isomers (α, β and γ) and the 3 phenolic BFRs (i.e. tetrabromobisphenol A (TBBPA), 6-OHBDE-47 and 2,4,6-tribromophenol (2,4,6-TBP)), and the dibromopropylether (DBPE) of TBBPA.

For the pre-screening of these BFRs, a battery of in-vitro assays (human, rat and fish cell lines) and QSAR models were used. The in-vitro pre-screening assays included (ant)agonism of estrogen receptor (ER), (ant)agonism of androgen receptor (AR), (ant)agonism of progesterone receptor (PR), (ant)agonism of dioxin receptor (DR), capacity to displace thyroxine (T4) from its plasma carrier protein transthyretin (TTR), triiodothyronine (T3) mimicking and inhibiting capacity, vitellogenin induction, androgen synthesis (CYP17 and 5a-reductase), aromatase (CYP 18) activity, estrogen sulfotransferase (ESULT) activity, CYP 1A1 activity, CYP 3A4 activity and cytotoxicity (MTT and LDH assays).

Based on the in-vitro pre-screening results, the test set of 27 compounds was roughly divided into 5 subgroups. HBCD (technical mixtures and the 3 individual isomers) was included in the group with low to moderate anti-androgenic and anti-progestagenic activity, low to moderate DR antagonistic activity and moderate to high T-Screen synergistic activity.

From an evaluating point of view, it was concluded that the selected battery of in-vitro bioassays allowed hazard profiling of the selected compounds, resulting in a recommendation of a small set of BFRs for further in-vivo testing. This shortlist was compared with a list of additional criteria. Based on these additional criteria, consensus was reached across the consortium (of the FIRE project) to select TBBPA, HBCD and a few other BFRs for in-vivo toxicity studies.

Under Themes 2 and 3, 2 sub-chronic (4-week repeated dose) studies enhanced for endocrinological parameters were completed with TBBPA and HBCD. These studies have been discussed in detail in Section 8.2.4.

Further, for the aquatic wildlife hazard identification and dose-response assessment, the in-vivo endocrine disrupting effects of the selected test BFRs, including HBCD, were studied in a freshwater lower vertebrate model species (zebrafish) as well as in an estuarine wildlife species (flounder), a common indicator species in monitoring programs. These studies are summarised in Sections 9.2.2.2 and 9.5, respectively. Overall, the results from the studies indicated limited endocrine effects of HBCD in zebrafish or flounder.

As part of the human and aquatic wildlife exposure assessment (Themes 4 and 5), human (breast milk) and environmental samples were analysed for HBCD (and other BFR) concentrations. HBCD (sum of isomers) was been determined in breast milk from Czechoslovakia and Norway. The levels in the Norwegian samples were much higher (n=49; median 0.63 ng/g lipid; range ~0.25–2.0 ng/g lipid) compared to the Czech samples (n=12; median: 0.11 ng/g lipid; range: <0.1–0.43 ng/g lipid). However, HBCD was detected only in a low number of breast milk samples.

Diet samples of The Netherlands showed detectable levels of PBDEs and HBCD. Based on consumption patterns, daily intakes of the HBCD were estimated as 1.17 ng/kg-bw/d. Assuming an average weight of adult between of 60–70 kg, these results are consistent with values calculated from food frequency data.

Samples of the food chains of tern, seal and polar bear were collected from the Western Scheldt, Wadden Sea, Elbe, Moldau, Mersey, Tees, Clyde, Oslofjord, Froan and Svalbard regions. HBCD was frequently found in all food chains. α-HBCD was the dominating isomer in biota and γ-HBCD was the dominating isomer in abiotic compartments.

Analysis of environmental samples showed that HBCD is the dominating BFR in the abiotic compartments of environment, Concentrations of PBDEs and HBCD are highest in the Western Scheldt estuary, followed by Oslofjord and Wadden Sea. Lowest concentrations are found in the Arctic. Higher levels of HBCD were also found in the Western Scheldt than in the Wadden Sea / North Sea.

The ability of fish and marine mammals to metabolise the frequently α, β and γ somers of HBCD and other BFRs was also evaluated by in-vitro biotransformation assays using hepatic microsomes of a variety of marine mammals and fishes. Microsomes were prepared from the livers of harbour seal, pilot whale and 7 fish species caught in the Marsdiep (Wadden Sea). Of the wide range of fish families, including benthic feeders, demersal, and pelagic fish, only microsomes of the flatfish species dab and flounder (*L. limanda* and *P. flesus*, respectively) were capable of metabolising all 3 isomers of HBCD.

Studies with hepatic microsomes from pre-induced rats with HBCD were carried out to provide information on the structure-transformation relationships that govern metabolism of these compounds. Of the different HBCD isomers, β-HBCD appears to be most rapidly metabolisable. In environmental residues, γ-HBCD dominates in the technical mixture (about 90%) and often also in sediments, but the α-isomer often dominates in residues of marine top predators and also in humans. In-vitro assays were performed with hepatic microsomes of pre-induced laboratory rat, harbour seal, and 4 fish species, showing that the α-isomer is more resistant to biotransformation by the cytochrome P450 system than β- and γ-HBCD. This explains the observed enrichment of α-HBCD in residues from marine organisms. However, α-HBCD can also be hydroxylated by the cytochrome P450 system in at least some fish species.

Degradation of HBCD was also studied. However, the analytical data showed no consistent trend in concentrations of HBCD isomers with time, making the results of this experiment rather inconclusive.

The report concluded that poybrominated flame retardants studied in the FIRE project show endocrine properties in in-vitro *and* in-vivo studies. For HBCD, data were consistent with previously reported data, especially with respect to thyroid system. The project provides useful data that can be used for risk assessment and monitoring. The report recommends that further monitoring of BFRs studied in FIRE is required. In addition, as a result of regulation of currently used flame retardants, the production and use of their alternatives may increase. Monitoring and research to support risk assessment of these alternative products needs serious attention and may need to be expanded.

Glossary

NICNAS uses the IPCS Risk Assessment Terminology (WHO, 2004) glossary, which includes Part 1: IPCS/OECD Key Generic Terms used in Chemical Hazard / Risk Assessment; and Part 2: IPCS Glossary of Key Exposure Assessment Terminology. The IPCS Risk Assessment Terminology can be accessed at http://www.who.int/ipcs/methods/harmonization/areas/ipcsterminologyparts1and2.pdf

Adverse effect	Change in the morphology, physiology, growth, development, reproduction, or life span of an organism, system or (sub)population that results in an impairment of functional capacity, an impairment of the capacity to compensate for additional stress, or an increase in susceptibility to other influences.
Assessment	Evaluation of appraisal of an analysis of facts and the inference of possible consequences concerning a particular object or process.
Assessment endpoint	Quantitative/qualitative expression of a specific factor with which a risk may be associated as determined through an appropriate risk assessment.
Chronic exposure	A continuous or intermittent long-term contact between an agent and a target. (Other terms, such as "long-term exposure," are also used.)
Concentration	Amount of a material or agent dissolved or contained in unit quantity in a given medium or system.
Dose	Total amount of an agent administered to, taken up or absorbed by an organism, system or (sub) population.
Dose-effect relationship	Relationship between the total amount of an agent administered to, taken up or absorbed by an organism, system or (sub) population and the magnitude of a continuously-graded effect to that organism, system or (sub)population Related terms: *effect assessment, dose-response relationship, concentration-effect relationship*.
Dose rate	Dose per unit time

Dose-related effect	Any effect to an organism, system or (sub) population as a result of the quantity of an agent administered to, taken up or absorbed by that organism, system or (sub) population.
Dose-response	Relationship between the amount of an agent administered to, taken up or absorbed by an organism, system or (sub) population and the change developed in that organism, system or (sub) population in reaction to the agent. Synonymous with *dose-response relationship*. Related Term: *dose-effect relationship, effect assessment, concentration-effect relationship*.
Dose-response curve	Graphical presentation of a dose-response relationship.
Dose-response relationship	Relationship between the amount of an agent administered to, taken up or absorbed by an organism, system or (sub) population and the change developed in that organism, system or (sub) population in reaction to the agent. Related Terms: *dose-effect relationship, effect assessment, concentration-effect relationship*.
Effect	Change in the state or dynamics of an organism, system or (sub) population caused by the exposure to an agent.
Exposure	Concentration or amount of a particular agent that reaches a target organism, system or (sub) population in a specific frequency for a defined duration.
Exposure assessment	Evaluation of the exposure of an organism, system or (sub) population to an agent (and its derivatives). Exposure assessment is the third step in the process of Risk Assessment.
Exposure concentration	The exposure mass divided by the contact volume or the exposure mass divided by the mass of contact volume depending on the medium.
Exposure duration	The length of time over which continuous or intermittent contacts occur between an agent and a target. For example, if an individual is in contact with an agent for 10 minutes a day, for 300 days over a one-year time period, the exposure duration is one year.

Exposure period	The time of continuous contact between an agent and a target.
Exposure route	The way an agent enters a target after contact (e.g. by ingestion, inhalation or dermal absorption).
Exposure scenario	A set of conditions or assumptions about sources, exposure pathways, amount or concentrations of agent(s) involved, and exposed organism, system or (sub) population (i.e. numbers, characteristics, habits) used to aid in the evaluation and quantification of exposure(s) in a given situation.
Fate	Pattern of distribution of an agent, its derivatives or metabolites in an organism, system, compartment or (sub) population of concern as a result of transport, partitioning, transformation or degradation.
Predicted Hazard	Inherent property of an agent or situation having the potential to cause adverse effects when an organism, system or (sub) population is exposed to that agent.
Hazard assessment	A process designed to determine the possible adverse effects of an agent or situation to which an organism, system or (sub) population could be exposed. The process includes hazard identification and hazard characterisation. The process focuses on the hazard in contrast to risk assessment, where exposure assessment is a distinct additional step.
Hazard characterisation	The qualitative and, wherever possible, quantitative description of the inherent properties of an agent or situation having the potential to cause adverse effects. This should, where possible, include a dose-response assessment and its attendant uncertainties. Hazard characterisation is the second stage in the process of hazard assessment, and the second step in Risk Assessment. Related terms: *dose-effect relationship, effect assessment, dose-response relationship, concentration-effect relationship.*
Hazard identification	The identification of the type and nature of adverse effects that an agent has inherent capacity to cause in an organism, system or (sub) population. Hazard identification is the first stage in hazard assessment and the first step in the process of Risk Assessment.

Intake	The process by which an agent crosses an outer exposure surface of a target without passing an absorption barrier, i.e. through ingestion or inhalation.
Margin of exposure	Ratio of the no-observed-adverse-effect level (NOAEL) for the critical effect to the theoretical, predicted or estimated exposure dose or concentration. Related term: *margin of safety*.
Risk assessment	A process intended to calculate or estimate the risk to a given target organism, system or (sub)population, including the identification of attendant uncertainties, following exposure to a particular agent, taking into account the inherent characteristics of the agent of concern as well as the characteristics of the specific target system. The Risk Assessment process includes four steps: hazard identification, hazard characterisation (related term: *dose-response assessment*), exposure assessment, and risk characterisation. It is the first component in a risk analysis process.
Risk characterisation	The qualitative and, wherever possible, quantitative determination, including attendant uncertainties, of the probability of occurrence of known and potential adverse effects of an agent in a given organism, system or (sub) population, under defined exposure conditions. Risk Characterisation is the fourth step in the Risk Assessment process.
Risk management	Decision-making process involving considerations of political, social, economic, and technical factors with relevant risk assessment information relating to a hazard so as to develop, analyse, and compare regulatory and non-regulatory options and to select and implement appropriate regulatory response to that hazard. Risk management comprises three elements: risk evaluation; emission and exposure control; and risk monitoring.
Source	The origin of an agent for the purposes of an exposure assessment.
Target	Any biological entity that receives an exposure or a dose (e.g. a human, human population or human organ).

Threshold	Dose or exposure concentration of an agent below which a stated effect is not observed or expected to occur.
Time-averaged exposure	The time-integrated exposure divided by the exposure duration. An example is the daily average exposure of an individual to carbon monoxide. (Also called *time-weighted average exposure*.)
Toxicity	Inherent property of an agent to cause an adverse biological effect.
Uptake (absorption)	The process by which an agent crosses an absorption barrier.

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