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HPV
DATA SUMMARY AND TEST PLAN
FOR
HEXABROMOCYCLODODECANE (HBCD)
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Brominated Flame Retardant Industry Panel (BFRIP)
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to reach the desired flame retardancy. Typical HBCD levels in EPS are 0.67% and in XPS 2.5%. At present, HBCD appears the only flame retardant with characteristics for these applications. Any other flame retardant would likely need higher load levels in the polystyrene foam.

A secondary, though important, application of HBCD is as a flame retardant for upholstery textiles. In this application, HBCD is applied to the back of the upholstery fabric encapsulated in a polymer. Typical HBCD levels in the polymer backcoat are 6-15%. The potential exposure and hazard to consumers associated with this use were reviewed recently by the U.S. National Research Council (*D. Gardner and B. Walker, Chair, Toxicological Risks of Selected Flame Retardants, 2000, National Academy Press, Washington, D.C.; <http://www.nap.edu>*). The review found that direct exposure to the consumer was likely to be minimal, that the hazard index was less than 1 for all exposure routes (e.g. not likely to pose a health hazard), and that no further research was needed for assessing consumer health risks from HBCD.

A very minor application for HBCD is in video or audio equipment housings where V-2 levels of flame retardancy are used. HBCD is not used to flame retard electronic housings (e.g. television sets or computers) that must meet the higher V-0 flame retardancy standard.

4.0 HBCD TOXICOLOGY DATA SUMMARY

4.1 COMPOSITION OF THE HBCD USED AS TEST ARTICLE IN BFRIP-SPONSORED STUDIES

The American Chemistry Council's Brominated Flame Retardant Industry Panel (BFRIP) sponsored a number of tests on HBCD. These tests were performed over the course of eight years. In all cases, the test article was a composite composed of equal parts of the members' commercial HBCD product. BFRIP members are Albemarle Corporation, Dead Sea Bromine Group and Great Lakes Chemical Corporation. Each test article was characterized in accordance with Good Laboratory Practices guidelines. The test article used in the various tests ranged from approximately 90 to 100% HBCD (Table 1). The impurities, when present, were those typically observed in the commercial product, e.g. tetrabromocyclododecene, isobutanol, and other unidentified compounds.

Tests performed from 2001-2003 typically utilized a test article of 99.9% purity (Table 1). The stereoisomer content of this test article was approximately 85% gamma, 9% alpha and 6% beta. Test article used in earlier years was comparable with a slightly lower gamma content (80% gamma, 8% alpha and 6% beta) (Table 1).

Table 1. Composition of HBCD used as test article in BFRIP-sponsored studies (All studies performed using a composite of equal parts of Albemarle Corporation, Dead Sea Bromine Group and Great Lakes Chemical Corporation commercial product).

Study	Year	Mean Isomer Content (Area %) (Purity)			Isomer Sum
		Alpha	Beta	Gamma	
Validation of Water Solubility Analytical Method					
	1997	8.5	6.0	79.1	93.6
Water Solubility	1997	8.5	6.0	79.1	93.6
Log Kow	1997	8.5	6.0	79.1	93.6
Vapor Pressure	1997	8.5	6.0	79.1	93.6
Fish LC50	1997	8.5	6.0	79.1	93.6
Daphnia EC50	1997	8.5	6.0	79.1	93.6
Freshwater Algae EC50	1997	8.5	6.0	79.1	93.6
Daphnia Chronic	1998	8.5	6.0	79.1	93.6
Fish Early Life Stage	2001	9.4	6.3	84.3	100
Fish Bioconcentration+	2000	6.4	4.5	79.1	90
Rat 28 Day	1996	8.5	6.0	79.1	93.6
Rat 90 Day	2001	8.9	6.6	84.5	99.99
Rat Developmental+	1999	6.4	4.5	79.1	90
Guinea Pig Maximization	1997	8.5	6.0	79.1	93.6
Mouse LLNA*	2003	8.68	6.12	85.19	99.99
Chromosome Aberration	1996	8.5	6.0	79.1	93.6
In vivo Micronucleus	2000	8.9	6.6	84.5	99.99
Earthworm Survival & Reproduction*	2002	8.68	6.12	85.19	99.99
Terrestrial Plant: Seedling Emergence and Growth*	2002	8.68	6.12	85.19	99.99
Sediment Organism*	2003	8.68	6.12	85.19	99.99
Ready Biodegradability	1996	8.5	6.0	79.1	93.6
Sludge Respiration Inhibition*	2003	8.68	6.12	85.19	99.99
Soil Microcosm Biodegradation*	2003	8.68	6.12	85.19	99.99
Sediment Microcosm Biodegradation*	2003	8.68	6.12	85.19	99.99
Water Solubility of Alpha, Beta, Gamma Isomers	2004	7.67	5.15	83.04	95.86
Marine Algae, EC50	2004	7.67	5.15	83.04	95.86

*Results from reanalysis using stabilized THF; Initial analysis indicated 5.8% alpha isomer, 19.3% beta isomer, and 74.9% gamma isomer.

+Impurities specified as Tetrabromocyclododecene 0.7%, Isobutanol 0.1%; Other Unknowns 9.2%

4.2 ENVIRONMENTAL FATE (BFRIP)

HBCD's measured and predicted environmental fate parameters are provided in Table 2.

HBCD is predicted to partition in the environment to soil and sediment (~98%) where it will bind extensively to organic carbon (estimated $K_{oc,soil} = 1.25 \times 10^5$) and to be essentially immobile in soil. Based on a release of 1,000 kg/hr to air, water and soil, the predicted partitioning is: air 0.0007%, water 2.1%, soil 40% and sediment 58% (*Level III Fugacity Model, EPIWIN V3.04, Syracuse Research Corporation*). Aerobic and anaerobic biodegradation in soil and sediment has been demonstrated in microcosms. Half-lives of 7-63 days (soil) and 1-32 days (sediment) have been demonstrated with the shortest half-lives in anaerobic systems. HBCD is not expected to volatilize from water based on its river and lake volatilization half-lives and air-water partition coefficient. HBCD is expected to partition from water to organic matter (biomass to water partition coefficient = 1×10^7) (*EPIWIN V3.04, Syracuse Research Corporation*). Sewage treatment plants are predicted to remove HBCD from the influent to a high degree (94% removal), but biodegradation in the treatment plant is not expected. Removal in treatment plants is via partitioning to sludge.

4.2.1 Photodegradation

No photodegradation study has been performed on HBCD. However, in the event HBCD were able to undergo photodegradation, this is not expected to be a significant route of environmental degradation due to its low vapor pressure (6.27×10^{-5} Pa at 21°C) that would preclude substantial levels in the air and its predicted negligible partitioning to this media (0.007%).

4.2.2 Stability in Water (Hydrolysis)

HBCD is not expected to undergo hydrolysis. In the event HBCD were subject to hydrolysis, this is not expected to be a significant route of environmental degradation due to its low water solubility (3.4 ug/L) and its predicted minimal partitioning to this media (2.1%).

4.2.3 Biodegradation: Closed Bottle Test For Biodegradability (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD guidelines and Good Laboratory Practices.

HBCD was tested for ready biodegradation in a 28-day 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. No biodegradation was observed; the percent biodegradation was 0 (*Schaefer, E and Haberlein, D., 1996, Hexabromocyclododecane (HBCD): Closed Bottle Test. Project No.: 439E-102. Wildlife International Ltd. Easton, MD*).

Table 2. Environmental Fate Parameters for HBCD.

Parameter	Estimation Program or Test Result	Result
Photodegradation	-	Not likely to be a significant route of environmental degradation due to low vapor pressure
Hydrolysis	-	Not likely to be a significant route of environmental degradation due to low water solubility
Transport	Calculated (EPIWIN QSAR; EUSES)	Atmospheric half life = 1.75 day Subcooled vapor pressure = 4.93×10^{-3} Pa
Distribution	Estimated (EPI win, V.3.04)	Level III Fugacity Model predicts at 1000 kg/Hr emissions to air, water and soil: Air 0.0007%, Water 2.1%, Soil 40%, Sediment 58%
Atmospheric Oxidation	Estimated (EPI win, V.3.04)	Overall OH Rate Constant = 5.0×10^{-12} cm ³ /molecule-sec Half-Life = 2.1 Days (12-hr day; $1.56 \times 10^{+6}$ OH/cm ³) Half-Life = 25.6 Hrs
Henry's Law Constant	Estimated (EPI win, V.3.04)	6.4×10^{-11} atm-m ³ /mole at 25 °C 2.6×10^{-9} unitless at 25 °C
Soil Koc	Estimated (EPI win, V.3.04)	$1.25 \times 10^{+5}$
Octanol-Water Partition Coefficient	Estimated (EPI win, V.3.04)	$5.4 \times 10^{+7}$
Air-Water Partition coefficient	Estimated (EPI win, V.3.04)	$2.6 \times 10^{+7}$
Biomass to Water Partition Coefficient	Estimated (EPI win, V.3.04)	$1.1 \times 10^{+7}$
Volatilization from Water	Estimated (EPI win, V.3.04)	Half life: 2,631 years (River); $2.8 \times 10^{+4}$ years (Lake)
Sewage Treatment Plant Fugacity Model Level III	Estimated (EPI win, V.3.04)	Total Removal: 94%, Total Biodegradation: 0.78%, Primary Sludge: 59.87%, Waste Sludge: 33.35%, Final Water Effluent: 6%
Fugacity Model	Estimated (EPI win, V.3.04)	At Emissions to Air, Water, Soil and Sediment of 1,000, 1,000, 1,000 and 0 kg/hr, respectively: Fugacity (atm): Air 9.9×10^{-15} , Water 2.7×10^{-18} , Soil 4.1×10^{-20} , Sediment 2.6×10^{-18} Reaction (kg/hr): Air 0.91, Water 97.7, Soil $1.9 \times 10^{+3}$, Sediment 686 Advection (kg/hr): Air 0.67, Water 203, Soil 0, Sediment 114 Reaction (%): Air 0.03, Water 3.3, Soil 63.3, Sediment 22.9 Advection (%): Air 0.02, Water 6.8, Soil 0, Sediment 3.8
Biodegradation	OECD, GLP (BFRIP 1996) OECD, GLP (BFRIP, Dow, 2003) OECD, GLP (BFRIP, Dow, 2003)	Not readily biodegradable Aerobic river sediment microcosms, $t_{1/2} = 11$ and 32 days Anaerobic river sediment microcosms, $t_{1/2} = 1.1$ and 1.5 days Aerobic soil microcosm, $t_{1/2} = 63$ days Anaerobic soil microcosm, $t_{1/2} = 6.9$ days

4.2.4 Activated Sludge Respiration Inhibition (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD (209) guidelines and Good Laboratory Practices.

HBCD's effect on the respiration of activated sludge microorganisms was assessed using control, reference and treatment groups. 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 ~ three hours, the respiration rates of the test solutions were measured using a dissolved oxygen meter. The individual respiration rates of the two controls were 60.5 and 55.5 mg O₂/L/hr. The difference between the two 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 percent inhibition was observed in the treatment group. (*Schaefer E and Siddiqui A. 2003. Hexabromocyclododecane (HBCD): An Activated Sludge, Respiration Inhibition Test. Project Number: 439E-108A. Wildlife International, Ltd. Easton, MD.*)

4.2.5 Transformation in Aerobic and Anaerobic Water/Sediment Microcosms (BFRIP and Dow Chemical Company)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to Good Laboratory Practices.

The transformation of hexabromocyclododecane (HBCD) was determined in aerobic and anaerobic water/sediment microcosms based on the Organization for Economic Co-Operation and Development (OECD) Test Guideline 308 "Aerobic and Anaerobic Transformation in Aquatic Sediment Systems." Laboratory batch microcosms were prepared with authentic water and sediment collected from two rivers in the eastern United States. Aerobic microcosms were pre-incubated at 20 ± 1°C for 49 days and maintained by periodically exchanging the headspace of the microcosms with ambient air to replenish oxygen. Anaerobic microcosms were prepared in an anoxic atmosphere (70% N₂, 28% CO₂, and 2% H₂). The microcosms were pre-incubated at 23 ± 1°C for 43 to 44 days to allow the microcosms to stabilize. HBCD was then added to the microcosms at nominal concentrations ranging from 34 to 89 ng/g (sediment dry weight). Biologically inhibited (i.e., abiotic) controls were prepared by steam sterilization of the sediment/water mixture prior to the addition of HBCD. Microcosms were incubated in the dark at 20 ± 1°C for 119 days. The concentration of HBCD in the microcosms was determined at selected time intervals in the water and sediment phases utilizing high performance liquid chromatography-mass spectrometry (LC-MS). Aerobic microcosms

were analyzed on days 0, 1, 7, 21, 64, 91, and 119, while anaerobic microcosms were analyzed on days 0, 1, 7, 14, 61 (or 62), 91, and 119.

HBCD concentrations decreased over time in both the aerobic and anaerobic microcosms. HBCD concentrations in the viable aerobic microcosms from both river systems decreased at least 90% within 21 days, while the corresponding decreases in the abiotic controls ranged from 7 to 62%. Disappearance of HBCD was observed in both the viable and abiotic anaerobic microcosms with the rate of loss more rapid in the viable microcosms, with HBCD reaching non-detected levels within 7 days. In contrast, HBCD concentrations in the abiotic controls decreased from 48 to 62% after 14 days. Pseudo-first order kinetics rate constants for the biotransformation of HBCD were determined by subtracting the abiotic rate constant from the viable rate constant. Biotransformation half-lives for HBCD in the two river systems were determined to be 11 and 32 days in the aerobic microcosms and 1.1 and 1.5 days in the anaerobic microcosms. Brominated degradation products were not detected in the sediment and water layers or in the headspace of the microcosms.

The purpose of this study was to determine the environmental lifetime of HBCD under realistic environmental conditions. Laboratory microcosms were used to evaluate the transformation of HBCD in aerobic and anaerobic water/sediment microcosms. Sediment degradation processes are expected to play a major role in determining the environmental lifetime of HBCD since, upon release into the environment, the majority of HBCD is likely to partition into the soil or sediment compartments. In this study, HBCD loss was observed in both viable and abiotic sediments although the rates were appreciable faster in the viable sediments. Biologically mediated transformation processes (*i.e.*, biotransformation) accelerated the rate of loss of HBCD when compared to the biologically inhibited (*i.e.*, heat-treated) microcosms. Brominated degradation products were not detected in any of the sediment microcosms.

Limited information is available for the reactions of HBCD in the environment. However, the aerobic degradation and mineralization of a similar type of cyclic aliphatic halogenated fire retardant, FR-651A (mixture of pentabromo-chlorocyclohexane, tetrabromo-dichlorocyclohexane, and tribromotrichlorocyclohexane) has been observed. A soil half-life of ~11 days, based upon disappearance of ^{14}C -FR-651A from soil, was reported. Complete degradation of ^{14}C -FR-651A was also observed with a mineralization half-life on the order of 93 days.

An examination of the reactions of brominated aliphatic compounds that contain structural features similar to HBCD may also provide insight into the possible reaction pathways available for HBCD. Brominated aliphatic compounds are known to be susceptible to both hydrolytic and nucleophilic attack. The reactivity of halogenated aliphatic compounds depends on the strength of the carbon-halogen bond, and increases in the order of $\text{F} < \text{Cl} < \text{Br}$. For example, the hydrolysis half-lives at pH 7 and 25°C for methyl fluoride, methyl chloride, and methyl bromide are 1.1×10^4 , 340, and 20 days, respectively. At environmental pH's, neutral and base catalyzed hydrolysis reactions are most important, and depend on the degree and patterns of halogen substitution. Sulfur

based nucleophiles can react rapidly with halogenated aliphatic compounds, thereby reducing their lifetimes in the environment. The half-life of 1-bromohexane in water at 25°C was reduced from 20 days to approximately one day in 5 mM HS⁻ or 0.07 mM polysulfide (S_x²⁻). Similarly, the half-life of 1,2-dibromoethane in water at 25°C was reduced from 1,000 days to 4 days in the presence of 5 mM HS⁻. Sulfide and polysulfides would be expected to be present in sediments at low redox potentials. Thus, reaction of HBCD with these sulfur-based nucleophiles may partly explain the loss of HBCD in the microcosm studies.

Since HBCD contains three pairs of vicinal bromine atoms, simple aliphatic compounds containing vicinal bromine atoms can provide insight into possible reaction pathways. For example, 1,2-dibromoethane reacts with nucleophiles via both substitution and elimination reactions. Reaction with HS⁻ via an “SN2” substitution reaction results in the formation HS-CH₂-CH₂-SH, while an elimination reaction results in the formation of H₂C=CHBr. A combination of elimination and substitution reactions can result in the formation of a mixture of HO-CH₂-CH₂-OH and H₂C=CHBr. Similar types of reactions may have occurred in this study. Note that the water from both the Schuylkill and Neshaminy Rivers contained sulfate at levels > 40 ppm. There is a high likelihood that sulfide like compounds were generated in the sediment microcosms, as these mixtures became anoxic.

Halogenated aliphatic compounds are susceptible to reductive dehalogenation reactions in natural reducing environments. The rates of reactions vary depending on the strength and electron affinity of the carbon-halogen bond, and the stability of the carbon-radical species resulting from the initial electron transfer that occurs. Vicinal dehalogenation reactions are particularly important, and are dependent on the halogen atom. Half-lives for vicinal dehalogenation of 1,2-diiodoethane, 1,2-dibromoethane, and 1,2-dichloroethane are 0.6, 55, and 1.8 x 10⁴ hours, respectively. The rapid disappearance of HBCD in the anaerobic sediment microcosms may be partly explained by reductive dehalogenation reactions. In addition, the disappearance of HBCD in the aerobic sediment microcosms may also be at least partly explained by reductive dehalogenation reactions. Anaerobic gradients often occur below the surface of sediments that are exposed to an aerobic water column. Such gradients would be expected to form in the static microcosms used in this study.

Based upon these results, HBCD is not persistent in sediment. (*Davis J, Gonsior S and Marty G. Evaluation Of Aerobic And Anaerobic Transformation Of Hexabromocyclododecane In Aquatic Sediment Systems. Study Number 021081. Environmental Chemistry Research Laboratory, Toxicology & Environmental Research and Consulting. The Dow Chemical Company Midland, Michigan. 2003.*)

4.2.6 Transformation in Aerobic and Anaerobic Soil Microcosms (BFRIP and Dow Chemical Company)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to Good Laboratory Practices.

The transformation of HBCD was determined in aerobic and anaerobic soils based on the Organization for Economic Co-Operation and Development (OECD) Test Guideline 307 "Aerobic and Anaerobic Transformation in Soil." Soil microcosms were prepared by adding a sandy loam surface soil to serum bottles sealed with Teflon[®] coated septa. Aerobic microcosms were prepared by adjusting the soil moisture to 20% (by weight) and periodically exchanging the headspace of the microcosms with ambient air to replenish oxygen. The microcosms were pre-incubated at $20 \pm 1^\circ\text{C}$ for 35 days. Anaerobic microcosms were prepared in an anaerobic atmosphere (70% N₂, 28% CO₂, and 2% H₂) by flooding the soil with water and pre-incubating the microcosms at $23 \pm 1^\circ\text{C}$ for 43 days to allow low redox (e.g., methanogenic) conditions to develop. HBCD was then added to microcosms at a nominal concentration of 25 ng/g (soil dry weight), together with activated sludge (5 mg/g, dry weight basis) from a municipal wastewater treatment plant to simulate sludge land treatment applications. Biologically inhibited (abiotic) controls were prepared by steam sterilization prior to the addition of HBCD. Microcosms were incubated in the dark at $20 \pm 1^\circ\text{C}$ for 119 days. The concentration of HBCD in the microcosms was determined at selected time intervals utilizing high performance liquid chromatography-mass spectrometry (LC-MS). Aerobic microcosms were analyzed on days 0, 1, 7, 21, 48, 65, and 119, while anaerobic microcosms were analyzed on days 0, 1, 7, 21, 56, 91, and 119.

HBCD concentrations decreased over time in both the aerobic and anaerobic soils. HBCD concentrations decreased 75% over 119 days in the viable aerobic soil microcosms, compared to a 3% decrease in the abiotic controls, indicating that biological processes were responsible for most of the losses observed. Under anaerobic conditions, HBCD concentrations decreased 92% over 21 days in the viable microcosms compared to a less than 1% decrease in the abiotic controls. Pseudo-first order kinetics rate constants for the biotransformation of HBCD were determined by subtracting the abiotic rate constant from the viable rate constant. Biotransformation half-lives for HBCD were determined to be 63 and 6.9 days in the aerobic and anaerobic soils, respectively. Brominated degradation products were not detected in the soil or in the headspace of the microcosms.

The purpose of this study was to determine the environmental lifetime of HBCD under realistic environmental conditions. Laboratory microcosms were used to evaluate the transformation of HBCD in aerobic and anaerobic soils. Soil degradation processes are expected to play a major role in determining the environmental lifetime of HBCD since, upon release into the environment, the majority of HBCD is likely to partition into the soil or sediment compartments. In this study, HBCD loss was observed in both viable and

abiotic soil microcosms although the rates were appreciably faster in the viable soils. Biologically mediated transformation processes (i.e., biotransformation) accelerated the rate of loss of HBCD when compared to the biologically inhibited (i.e., heat-treated) soils. No brominated degradation products were observed in either system.

Limited information is available for the reactions of HBCD in the environment. However, the aerobic degradation and mineralization of a similar type of cyclic, aliphatic halogenated fire retardant, FR-651A (mixture of pentabromochlorocyclohexane, tetrabromo-dichlorocyclohexane, and tribromotrichlorocyclohexane) was observed. A soil half-life of ~11 days based upon disappearance of ^{14}C -FR-651A from soil was reported. Complete degradation of ^{14}C -FR-651A was also observed with mineralization half-life on the order of 93 days.

An examination of the reactions of brominated aliphatic compounds that contain structural features similar to HBCD can also provide insight into the possible reaction pathways available for HBCD. Brominated aliphatic compounds are known to be susceptible to both hydrolytic and nucleophilic attack. The reactivity of halogenated aliphatic compounds increases in the order of $\text{F} < \text{Cl} < \text{Br}$. For example, the hydrolysis half-lives at pH 7 and 25 °C for methyl fluoride, methyl chloride, and methyl bromide are 1.1×10^4 , 340, and 20 days, respectively. At environmental pH's, neutral and base catalyzed hydrolysis reactions are most important, and depend on the degree and patterns of halogen substitution.

Sulfur based nucleophiles can react rapidly with halogenated aliphatic compounds, thereby reducing their lifetimes in the environment. The half-life of 1-bromohexane in water at 25 °C was reduced from 20 days to approximately one day in 5 mM HS^- or 0.07 mM polysulfide (S_x^{2-}). Similarly, the half-life of 1,2-dibromoethane in water at 25 °C was reduced from 1,000 days to 4 days in the presence of 5 mM HS^- . Sulfide and polysulfides would be expected to be present in soils at low redox potentials. Thus, reaction of HBCD with these sulfur-based nucleophiles may partly explain the loss of HBCD in the soil studies.

Since HBCD contains three pairs of vicinal bromine atoms, the transformation of simple aliphatic compounds containing vicinal bromine atoms may provide insight into possible reaction pathways for HBCD. For example, 1,2-dibromoethane reacts with nucleophiles via both substitution and elimination reactions. Reaction with HS^- via an "SN2" substitution reaction results in the formation of $\text{HS-CH}_2\text{-CH}_2\text{-SH}$, while an elimination reaction under alkaline conditions results in the formation of $\text{H}_2\text{C=CHBr}$. A combination of elimination and substitution reactions can result in the formation of a mixture of $\text{HO-CH}_2\text{-CH}_2\text{-OH}$ and $\text{H}_2\text{C=CHBr}$. Similar mechanisms may be responsible for the loss of HBCD observed in this study.

Halogenated aliphatic compounds are susceptible to reductive dehalogenation reactions in natural reducing environments. The rates of reactions vary depending on the strength and electron affinity of the carbon-halogen bond and the stability of the carbon-radical species resulting from the initial electron transfer that occurs. Vicinal dehalogenation reactions are particularly important, and are dependent on the halogen atom. Half-lives

for vicinal dehalogenation of 1,2-diiodoethane, 1,2-dibromoethane, and 1,2-dichloroethane are 0.6, 55, and 1.8×10^4 hours, respectively. The rapid disappearance of HBCD in the anaerobic soil microcosms may be partly explained by reductive dehalogenation reactions.

Based upon these results, HBCD is not persistent in soil. (Davis J, Gonsior S and Marty G. 2003. *Evaluation Of Aerobic And Anaerobic Transformation Of Hexabromocyclododecane In Soil. Study Number 021082. Environmental Chemistry Research Laboratory. Toxicology & Environmental Research and Consulting. The Dow Chemical Company. Midland, MI.*)

4.2.7 Investigation of the Biodegradation of [¹⁴C]Hexabromocyclododecane in Sludge, Sediment, and Soil (European Brominated Flame Retardant Industry Panel)

This study investigated the biodegradation of the three HBCD stereoisomers, alpha, beta and gamma, and the identity of major degradation products. The formation and identification of degradation products of HBCD was assessed in activated and digester sludge, river sediment, and surface soil. Both aerobic and anaerobic biodegradation were evaluated in laboratory reaction mixtures and batch microcosms. This study was performed according to the relevant OECD Guidelines (302B; 307, 308), ISO 11734, and Good Laboratory Practices.

To generate sufficient levels of [¹⁴C]degradation products for their identification, [¹⁴C]HBCD was added to reaction mixtures and microcosms at nominal concentrations ranging from 3 to 5 mg/kg (or mg/L), exceeding the water solubility of the test material by greater than an order of magnitude. Duration of the studies ranged from approximately 60 to 112 days. Reaction mixtures and batch microcosms were extracted and analyzed by high performance liquid chromatography (HPLC) with radiochemical detection to follow the degradation of the 3 stereoisomers and formation of [¹⁴C]products. Product identification was facilitated by analyses of extracts from the soil, sediment and sludge mixtures by HPLC-atmospheric pressure photo ionization-mass spectrometry (APPI-MS) or gas chromatography-electron impact ionization-mass spectrometry (GC-EI-MS).

Substantial biological transformation of [¹⁴C]HBCD was observed in the anaerobic digester sludge and in freshwater aerobic and anaerobic sediment microcosms. Conversely, no degradation of HBCD was noted in the soil microcosms incubated under aerobic conditions. In the digester sludge and sediment, degradation of each of the three stereoisomers occurred over the course of the study. Little difference was noted in the disappearance of the three stereoisomers, indicating similarity in the extent of degradation of each isomer.

Concomitant with the loss of [¹⁴C]HBCD in the sludge and sediment test mixtures was formation of three [¹⁴C]products. Using a combination of HPLC-APPI-MS and GC-EI-MS these metabolites were identified as tetrabromocyclododecene, dibromocyclododecadiene, and cyclododecatriene. These products suggest HBCD is

sequentially debrominated via dihaloelimination by naturally occurring microorganisms in wastewater sludge and aquatic sediment. During each sequential debromination, two bromines are lost from vicinal carbons with the subsequent formation of a double bond between the adjacent carbon atoms. These results demonstrate microorganisms naturally occurring in aquatic sediment and wastewater sludges can completely debrominate HBCD. (Davis JW, Gonsior SJ, Markham DA, and Marty GT. 2004. *Investigation of the biodegradation of [14C]hexabromocyclododecane in sludge, sediment, and soil. Laboratory Project Study ID 031178. Toxicology & Environmental Research and Consulting. The Dow Chemical Company, Midland, MI.*)

4.2.8 Transport (Fugacity) (BFRIP)

If released in equal amounts to air, water and soil, HBCD was predicted to partition to soil and sediment. Based on a release of 1,000 kg/hr to air, water and soil, the predicted partitioning would be: air – 0.0007%, water - 2.1%, soil - 40%, and sediment - 58%. The majority (86%) would be reacted in sediment (63%) and soil (23%) with only 11% of the total undergoing advection (*Level III Fugacity Model, EPIWIN modeling software, V3.04, Syracuse Research Corporation*).

4.2.9 Environmental Monitoring Data: Results Of Isomer-Specific Analysis Available As Of May 2004

Hexabromocyclododecane (HBCD) is used to flame retard extruded and expanded polystyrene that is then used as thermal insulation in buildings. A minor use is in upholstery textiles where it is applied as a backcoat on the fabric. The commercial HBCD product is composed of three stereoisomers that are designated alpha, beta and gamma in deference to their respective elution from a reverse phase column. The isomer content of the commercial product is typically 80-85% gamma, 8-9% alpha and 6% beta. The major impurity is tetrabromocyclododecene.

A laboratory fish bioconcentration study showed that the three stereoisomers were present in rainbow trout in rough approximation to that of the commercial product used as test article. A rat 90-day subchronic study on the commercial product showed that the alpha isomer predominated in adipose tissue with lesser quantities of the gamma and beta isomers. Given these apparent differences between species and isomers, the literature and unpublished data were reviewed for environmental monitoring studies that included HBCD (Table 3). Only those studies that utilized analytical methods allowing detection of the three HBCD isomers were included. Studies reporting only 'total HBCD' content were discarded. Results were separated by matrix: sewage treatment plants, sediment, landfill leachate, whole organisms, and specific tissues. The resulting dataset, both as a whole and for any specific matrix/organism, is small. Thus, caution should be used to guard against over interpretation of the results.

Sewage Treatment Plants (STP). STPs appear to do a good job removing HBCD from the influent. Plants with detectable amounts of HBCD in the influent typically had no detectable HBCD in the effluent. HBCD appears to settle in sludge; the gamma

isomer typically represented some 50% of the total. Sludge from the UK and Ireland typically contained all three isomers, but only one STP in the Netherlands had detectable levels of the beta isomer in sludge. For the 5 UK plants with measured influent, 1 had no detectable HBCD in the influent, 3 had gamma but no alpha or beta in the influent, and 1 had all 3 isomers with beta predominating. In the Netherlands, for the 5 plants measured, 3 had alpha in the influent but not beta or gamma, one had predominantly gamma with lesser alpha, and one had no detectable HBCD.

Conclusion: STPS perform their function in removing HBCD from the influent. Effluents from plants receiving HBCD typically contained no detectable HBCD. Gamma typically made up approximately 50% of the total in sludge; e.g. the gamma predominated. The predominant isomer in the influent appears to vary with country (gamma in UK, alpha in the Netherlands)/ however, the dataset is extremely small and variable so this should be viewed with caution.

Sediment. In those sediments with detectable HBCD, the gamma isomer was detected with greatest frequency (9 out of 27 sediment samples from the European Scheldt and Dutch Rivers had gamma as the predominant isomer and many of these had detectable levels of gamma only). Two sediments out of 27 had roughly equal levels of gamma and beta, one had roughly equal levels of alpha and gamma, and one had alpha only.

Conclusion - the HBCD isomer most often detected in sediment was gamma.

Landfill Leachate. Samples from the UK and Ireland had no detectable HBCD. Eleven samples of landfill leachate originating in the Netherlands were analyzed. Four contained no detectable HBCD. In the seven where HBCD was detected, the gamma isomer was the predominant and most frequently detected isomer. The beta isomer was detected in only one of the samples from the Netherlands.

Whole Organisms. Both alpha and gamma were detected in all Lake Ontario trout analyzed (5/5). Beta was not detected in any. Composites of 3 different feeder fish from Lake Ontario also had detectable levels of both alpha and gamma, but no beta. Composites of 3 invertebrates, analyzed separately by species, from Lake Ontario also had detectable levels of both alpha and gamma, but no beta. Alpha was present in the highest quantities in all Lake Ontario organisms. North Sea whelk, sea star and hermit crabs had no detectable levels of HBCD.

Conclusion - The alpha and gamma isomers were detected with the same frequency in freshwater species of Lake Ontario. The alpha isomer typically represented approximately 80% of the total. Analysis of whole organism, as opposed to individual tissues (see below), appears to generate the most consistent data.

Specific Tissues.

Liver, bird. The alpha isomer was most frequently detected (and the predominant isomer) in cormorant liver (n=5).

Liver, marine mammal. One marine mammal liver contained alpha but not beta or gamma, and one contained no detectable HBCD (n=2). Thus, no conclusion can be suggested.

Muscle, fish. The dataset for Whiting is too small and variable to draw any conclusion. Whitefish (Switzerland) contained alpha, but no beta or gamma.

Muscle, bird. The alpha, beta and gamma isomers were detected with roughly equal frequency.

Egg, bird. The alpha isomer was most frequently detected. The numbers of eggs with detectable quantities of beta and gamma were roughly equal.

Blubber. The alpha isomer was the most frequently detected and typically in the highest quantity. Only one sample out of 11 had detectable amounts of all 3 isomers.

Eel. The alpha isomer was the most frequently detected and typically made up the largest proportion of the total. However, a reasonable number of samples also contained gamma, and in few cases, the alpha and gamma levels were approximately equal.

Conclusion. This dataset is extremely small and highly variable. Continued analysis of liver, muscle and eel does not appear justified. Egg and blubber may be good indicators for birds and marine mammals, and thus continued analysis of these tissues may prove useful.

Textile Plants Located in Europe. The gamma isomer was the predominant HBCD isomer detected in waste water from 4 of 5 textile plants. In 1 of these 5 plants, alpha and gamma were present in approximately equal quantities. The gamma isomer predominated in soil surrounding the 5 plants.

Summary. The published literature and unpublished data were reviewed for environmental monitoring results pertaining to HBCD. Only those studies that included specific analysis for the alpha, beta and gamma HBCD stereoisomers were included. Environmental matrixes reporting results for all three stereoisomers included sewage treatment plants, sediment, whole organisms, specific tissues, and textile plant wastewater, and textile plant soil. The gamma isomer predominated in sewage treatment plant sludge, sediment, landfill leachate, textile plant wastewater and textile plant soil. The alpha isomer was the predominant isomer detected on the analysis of whole organisms (lake trout, feeder fish, 3 freshwater invertebrates). Analysis of individual tissues produced varying results. Of the individual tissues analyzed, avian egg and marine mammal blubber appeared to produce the most consistent results. The alpha isomer was the most frequently detected isomer in both. Continued monitoring of egg and blubber may be of value, but other tissue types are not recommended. The most consistent data is apparently generated on the basis of whole organism analysis. Sewage treatment plants are effective in removing HBCD from the influent - effluents from plants receiving HBCD typically contained no detectable HBCD.

Table 3. Environmental monitoring data: results of HBCD isomer-specific analysis available as of May 2004.

A. HBCD ISOMER SPECIFIC ANALYSIS IN DIFFERENT TISSUES¹

<u>Liver (All ppb ww)</u>	<u>alpha</u>	<u>beta</u>	<u>gamma</u>	<u>% Lipid, n</u>
Cormarant (ug/kg ww)	21	3	2	% Lipid not stated
	6	1	2	
	33	1	1	
	7	0.3	0	
	2	0	0	
H. Porpoise (ng/g ww)	2400	0	0	36% lipid, n=1
H. Seal (ng/g ww)	0	0	0	2% lipid, n=2 or 3
<u>Muscle or Fillet (All ppb ww)</u>				
Fish				
Whiting, UK (ug/kg ww)				
	506	247	283	% Lipid not staed
	81	61	149	
	0	0	0	
Whiting, Netherlands (ng/g ww)				
	0	0	0	0.6% Lipid, n=3
Whitefish, Switzerland, (ng/g lw)				
	210	0	0	2.6%, pool of 10
	100	0	0	3.8%, pool of 10
	66	0	0	1.5%, pool of 10
	54	0	0	7.2%, pool of 10
Avian				
Sparrow Hawk (ug/kg ww)				
	61	0	0	2.6% Lipid
	0	8.1	19	1.3
	0	9.4	11	2.2
	2.6	0	0	3.1
	13	26	150	1
	0	7.1	0	0.8
	4.8	0	0	1
	2.6	0	0	1.1
	0	9.2	0	1
	0	0	0	0 – 8.5% LIPID, n=60
<u>Egg, Avian (ug/kg ww)</u>				

¹ Data from draft EU Risk Assessment 2003, de Boer's bird study 2004, Gerecke 2003

Falcon	38	0	0	7
	72	0	0	11.1
	22	0	0	7.7
	0	9.2	0	0.8
	13	0	0	7.3
	5.1	0	0	7.2
	0	3.4	4.6	6.4
	28	0	0	5.5
	30	13	12	7.1
	0	8.9	0	6.3
	15	0	5.5	6.3
	27	0	0	4.2
	20	0	0	5.0
	0	0	0	0.8-17.1; n=40
<u>Blubber (All ppb ww)</u>				
Porpoise (ug/kg ww)	298	302	317	% Lipid not stated, n=5 f
	315	2	5	
	53	1	0	
	89	0.4	0	
	0	0	0	
H. Porpoise (ng/g ww)	3500	0	0	90% Lipid, n=4
	410	0	0	
	6400	0	0	
	800	0	0	
H. Seal (ng/g ww)	2000	0	0	70% Lipid, n=2
	0	0	0	
<u>Eel (whole body) (ug/kg ww)</u>				
	0	0	0	
	0	0	0	
	0	0	0	
	0	0	0	
	0	0	0	
	0	0	0	
	0	0	0	
	0	0	0	
	25	5	11	
	25	0	8	

5	0	0
0	0	0
22	21	1
2	0	0
5	0	0
3	0	0
0	0	0
0	0	0
52	0	12
36	0	8
28	0	7
8	0	0
14	0	0.4
17	0	15
2	0	0
4	0	0
8	0	10

B. HBCD ISOMER SPECIFIC ANALYSIS OF WHOLE ORGANISMS²

	Alpha	Beta	Gamma	%Lipid, n
Lake Trout, Lake Ontario, whole fish (ng/g ww), n=5				
	1	0	0.3	11 % lipid
	0.4	0	0.07	14% lipid
	1.2	0	0.26	9 % lipid
	0.6	0	0.1	15 % lipid
	3.8	0	0.7	14% lipid
Rainbow Smelt, Lake Ontario, composites of whole fish (ng/g ww)				
	0.3	0	0.04	1.4% lipid, n=5 fish
	0.2	0	0.04	1.6% lipid, n=5 fish
	0.2	0	0.03	1% lipid, n=20 fish
Slimy sculpin, Lake Ontario, composites of whole fish (ng/g ww)				
	0.1	0	0.02	0.9, n=15
	0.4	0	0.1	3%, n=10
	0.4	0	0.1	2%, n=10
Alewife, Lake Ontario, composites of whole fish (ng/g ww)				
	0.15	0	0.02	3.5% lipid, n=5
	0.12	0	0.01	5.5% lipid, n=5
	0.08	0	0.01	1.5% lipid, n=5

² Data from Tomy et al 2004 re Lake Ontario, and de Boer re North Sea.

Mysis, Lake Ontario, composite of whole organisms (ng/g ww)				
	0.07	0	0.02	3.1 % lipid, n>100
	0.04	0	0.01	3.8% lipid, n>100
Diporeia, Lake Ontario, composite of whole organisms (ng/g ww)				
	0.06	0	0.03	1.7%lipid, n>100
	0.05	0	0.02	1%lipid, n>100
Plankton, Lake Ontario, composite of whole organisms (ng/g ww)				
	0.04	0	0.03	0.6% lipid
	0.02	0	0	0.2% lipid
Whelk, North Sea (ng/g ww)				
	0	0	0	2.4%
	0	0	0	1.8
	0	0	0	1.5
Sea Star, North Sea (ng/g ww)				
	0	0	0	3.5%
	0	0	0	7.5
	0	0	0	7.6
Hermit Crab, North Sea (ng/g ww)				
	0	0	0	7.4%
	0	0	0	6.7
	0	0	0	8.5
	0	0	0	10.6
	0	0	0	9
	0	0	0	7.3
	0	0	0	9.5
	0	0	0	14.1
	0	0	0	9.8

C. HBCD Isomer Specific Analysis: Sewage Treatment Plants³

	Alpha	Beta	Gamma	
Burnham, UK				
Influent dissolved phase	7.9	12.5	3.2	ng/L
Influent particulate phase	0	29.4	0	ug/kg dw
Sludge	132	458	666	ug/kg dw
Effluent dissolved phase	0	0	0	ng/L
Effluent particulate phase	0	0	0	ug/kg dw
Latchingdon, UK				
Influent dissolved phase	0	0	9.1	
Influent particulate phase	0	0	2.3	
Sludge	205	321	432	
Effluent dissolved phase	0	0	0	
Effluent particulate phase	0	0	0	

³ All data from draft EU Risk Assessment.

Wickford, UK				
Influent dissolved phase	0	0	4.6	
Influent particulate phase	0	0	0	
Sludge	89.6	112	329	
Effluent dissolved phase	0	0	0	
Effluent particulate phase	0	0	0	
S. Woodham Ferrers, UK				
Influent dissolved phase	0	0	0	
Influent particulate phase	0	0	0	
Sludge	233	547	798	
Effluent dissolved phase	0	0	0	
Effluent particulate phase	0	0	0	
Chelmsford, UK				
Influent dissolved phase	0	0	4.3	
Influent particulate phase	0	0	0	
Sludge	541	897	1245	
Effluent dissolved phase	0	0	0	
Effluent particulate phase	0	0	0	
STP1, Netherlands				
Influent	670	0	0	ng/L
Sludge	0	0	0	ug/kg dw
Effluent	0	0	0	ng/L
STP2, Netherlands				
Influent	3800	0	0	
Sludge	0	0	48	
Effluent	9	0	8.7	
STP3, Netherlands				
Influent	40	0	0	
Sludge	15	0	5.4	
Effluent	0	0	5.1	
STP4, Netherlands				
Influent	75	0	180	
Sludge	440	120	760	
Effluent	0	0	0	
STP5, Netherlands				
Influent	0	0	0	
Sludge	-	-	-	
Effluent	1.4	0	0	
STP6, Netherlands				
Sludge	7.6	0	20	
STP7, Netherlands				
Sludge	3.5	0	3.6	
STP8, Netherlands				
Sludge	7.4	0	13	
STP9, Netherlands				
Sludge	0	0	0	
STP10, Netherlands				
Sludge	0	0	0	
Portlaoise, Ireland				
Sludge	280	349	604	
Sludge	372	523	750	
Clonmel, Ireland				
Sludge	3.9	0	149	
Sludge	10.1	0.13	258	
Cork, Ireland				
Sludge	2300	1800	3410	

Data from (de Boer *et al.*, 2002) as reported in draft EU Risk Assessment

Population equivalents:

STP 1-4, 7 and 10 high treatment capacity, 200 000 - 750 000

STP 5, 6 and 8 100 000 – 150 000

UK varying from 4 750, Latchingdon, to 143 000, Chelmsford.

D. HBCD ISOMER SPECIFIC ANALYSIS OF SEDIMENT⁴ (ug/kg ww)

Scheldt Basin (Europe)

	Alpha	Beta	Gamma
Warmebeek Achel-kluis	0	0	0
Moervaart Daknam	0	0	0
Benede Nete Duffel	0	0	0.5
Grote Beverdijk Lo-R.	0	0	0
Ijzer Nieuwpoort	0	0	0.9
Durne Lokeren	0	0	0
Leie Wervik	0	0	0
Leie Wevelgem	0	0	0
Leie Oselgem	0	0	0
Leie St Martens	7	0	31
Scheldt Doel	0	0	0
Scheldt Grens	0	0	82
Scheldt Oudenaarde	180	60	710
Antwerp Kruisschansbr.	0	0	0
Scheldt Kastel	0	0	5
Scheldt Kennedyt.	0.3	0	0
Dender Appels	0.3	0	0
Dender Ninove	0	0	0.2

Dutch Rivers (Europe)

Waal Tiel	0	0	0
Rhine Lobith	0	4	4
Hollans Diep	0	0	10
Haringvliet West	0	0	6
Haringvliet East	0	0	0
Nieuwe Mervede	2	0	1
Meuse Eijsden	0	0	0

⁴ All data from draft EU Risk Assessment.

Meuse Keizersveer	3	0	0
Roer Vlodrop	0	0	0

Data from (de Boer *et al.*, 2002) as reported in draft EU RA

UK	42	16-980	12-550	ppb dw
Ireland	0.3	0	0-30	
Dublin Bay, Ireland	0	0-1	0-11	
	0	0	0	
Norway	0-9	0-4	0-79	ng/kg ww

E. HBCD ISOMER SPECIFIC ANALYSIS OF LANDFIL LEACHATE⁵

	Alpha	Beta	Gamma	
UK	0	0	0 ng/L	(dissolved)
UK	0	0	0 ng/L	(particulate)
Netherlands	0-7000	0-13	0-36000	ug/kg dw
Ireland	0	0	0 ng/L	(dissolved)
Ireland	0	0	0 ng/L	(particulate)

⁵ All data from draft EU Risk Assessment.

4.3 ECOTOXICOLOGY DATA

HBCD was not acutely toxic to fish, daphnia or freshwater or marine alga at the limits of the gamma isomer's water solubility. HBCD was not chronically toxic to daphnia nor was it toxic to fish early life stages at the limits of the gamma isomer's water solubility. HBCD was bioconcentrated in fish, but not in earthworms. HBCD did not adversely effect the emergence or growth of six terrestrial plant species, and was not toxic to a sediment organism over a prolonged exposure. HBCD did not adversely effect the survival of earthworms, but did impact reproduction. A NOEC for this effect (reproduction in earthworms) was determined.

HBCD's water solubility was measured in 1997 using the generator column/column elution method, and determined to be 3.4 ug/L at 25°C (*J. Stenzel and B. Markley. 1997. Hexabromocyclododecane (HBCD): determination of the water solubility. Wildlife International LTD. Project Number: 439C-105. Wildlife International LTD. Easton, MD*). The analytical method, HPLC using a UV detector, was validated prior to use (*Kendall and Nixon. Analytical method verification for the determination of hexabromocyclododecane (HBCD) in well water. Final Report. Wildlife International LTD. Project Number: 439C-107. Wildlife International LTD, Easton, MD*). This method, the most sensitive available at the time of study performance, was capable of quantitating the gamma isomer, only. UV-active substances, whose peaks could not be resolved, interfered with quantifying the alpha and beta content. The solubility of HBCD was therefore based on that of the gamma isomer, which comprised ~80% of the test article.

The results of this water solubility study were used to set dose levels for aquatic studies performed between 1997 and 2001, and sponsored by ACC Brominated Flame Retardant Industry Panel.

In late 2003, further studies were undertaken to determine HBCD's potential effects on the marine algae, *Skeletonema*, in order to clarify results reported in Walsh et al., 1987. Preliminary work for the 2003 algae study (Desjardins D, MacGregor J and Krueger H. 2003. Draft Report: Hexabromocyclododecane (HBCD): A 72-Hour Toxicity Test with the Marine Diatom (*Skeletomema costatum*). Wildlife International Project Number: 439A-125. Wildlife International, Ltd. Easton, MD investigated whether HBCD exposures in marine media would be best conducted utilizing the water accommodated fraction (WAF) or eluates produced from a generator column. The analytical method utilized was HPLC/MS, an evolution of the original method that allowed quantification of all 3 isomers. The WAF preparations had serious problems with HBCD particulates that could not be resolved in a consistent manner by filtering or centrifugation. Thus, it was concluded the WAF method was not appropriate. The HBCD eluate from the generator column solved the particulate problem, but also revealed that the water solubility of the alpha and beta isomers was significantly different than that of the gamma isomer. The water solubility of the gamma isomer in the marine media was nearly equivalent to that determined in 1997 with reagent water. The solubility of the alpha and

beta isomers was approximately 47 and 16 ug/L, respectively. A standard GLP solubility study (MacGregor J and Nixon W. 2004. *Final Report. Determination of Hexabromocyclododecane (HBCD) Diastereomers using a Generator Column Method. Wildlife International Project Number: 439C-138. Wildlife International, Ltd. Easton, MD*) using reagent water confirmed these results. Thus, under the conditions of the generator column, HBCD's total water solubility (expressed as the sum of the three isomers) was 65.6 ug/L, and that of the gamma, alpha and beta isomers was 2.08, 48.8 and 14.7 ug/L, respectively.

4.3.1 Acute Toxicity to Fish

4.3.1.1 96-Hour Acute Toxicity Test With Rainbow Trout (*Oncorhynchus mykiss*) (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD guidelines and Good Laboratory Practices.

HBCD was not acutely toxic to rainbow trout at the limit of the gamma stereoisomer's solubility. HBCD's 96 hour LC50, no mortality concentration and no observed effect concentration were all > than the gamma stereoisomer's water solubility. The highest nominal dose tested was twice that water solubility. Nominal test concentrations were 0, 1.5, 2.2, 3.2, 4.6 and 6.8 ug/L and corresponded to mean measured concentrations (HPLC with UV/VIS detector) of 0, 0.75, 1.5, 2.3, 2.3 and 2.5 ug/L, respectively (Graves, W and Swigert, J. (1997) *Hexabromocyclododecane (HBCD): A 96-Hour Flow-Through Acute Toxicity Test with the Rainbow Trout (Oncorhynchus mykiss). Project Number: 439A-101. Wildlife International LTD, Easton, MD*).

4.3.1.2 Other Studies

The lack of acute toxicity in rainbow trout at HBCD's limit of water solubility is consistent with earlier studies performed at substantially higher concentrations. A Velsicol study in 1975 reported that the LC50 (96 Hr) in Bluegill sunfish (*L. macrochirus*) was >100 mg/L (nominal). A BASF study reported that the 96 hr LC50 in Golden orf (*L. idus*) was >10,000 mg/L (nominal).

4.3.1.3 Acute Toxicity to Aquatic Invertebrates: 48-Hour Acute Toxicity Test With *Daphnia magna* (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD guidelines and Good Laboratory Practices.

HBCD was not acutely toxic to *Daphnia magna*. HBCD's 48 hour EC50, no mortality/immobility concentration, and no observed effect concentration (6.8 ug/L nominal) in *Daphnia magna* were all > than gamma HBCD's water solubility (3.4ug/L

measured). The highest nominal dose tested was twice gamma HBCD's water solubility. Nominal test concentrations were 0, 1.5, 2.2, 3.2, 4.6 and 6.8 ug/L which corresponded to mean measured concentrations (HPLC with UV/VIS detector) of 0, 2.4, 1.8, 2.1, 2.3 and 3.2 ug/L, respectively (*Graves W and Swigert J. (1997) Hexabromocyclododecane (HBCD): a 48-hour flow-through acute toxicity test with the cladoceren (Daphnia magna). Project Number: 439A-102. Wildlife International Ltd., Easton, MD).*

4.3.2 Toxicity to Aquatic Plants

4.3.2.1 96-Hour Acute Toxicity Test With The Freshwater Alga (*Selenastrum capricornutum*) (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD guidelines and Good Laboratory Practices. This study was performed to complete the EU base set.

HBCD was not acutely toxic to *Selenastrum capricornutum*. HBCD's 96 hour EC10, EC50, EC90 and no observed effect concentration were all > than HBCD's water solubility based on the gamma isomer. The highest nominal dose tested was twice gamma HBCD's water solubility. Dose levels were 0, 1.5, 2.2, 3.12 4.6 and 6.8 ug/L (nominal). The mean measured concentration (HPLC with UV/VIS detector) at the 6.8 ug/L dose was 3.7 ug/L (*Roberts C. and Swigert J. Hexabromocyclododecane (HBCD): A 96-Hour Toxicity Test with the Freshwater Alga (Selenastrum capricornutum). Wildlife International Ltd. Project Number: 439A-103. June 3, 1997. Wildlife International Ltd., Easton, MD).*

4.3.2.2 Marine Alga

Walsh et al. 1987 (*Ecotoxicology and Environmental Safety, 14, 215-222*) reported testing the effect of media and test chemicals on acute toxicity in marine algae. HBCD was tested in 3 species of marine algae, and was not toxic at the limits of its water solubility. The EC50's are as follows: *Chlorella sp* 96 hr EC50 > water solubility (>1500ug/L); *S. costatum* 72 hr EC50 > water solubility (9.3-12 ug/L); *T. pseudonana* 72 hr EC50 > water solubility (50-370 ug/L).

4.3.3 Prolonged Exposure to Aquatic Organisms

HBCD was not toxic to fish early life stages or daphnia when exposed for prolonged periods of time. HBCD was bioconcentrated in fish.

4.3.3.1 Fish Early Life Stage In Rainbow Trout (*Oncorhynchus mykiss*) (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current OECD guidelines and Good Laboratory Practices.

Rainbow trout embryos were exposed to nominal HBCD water concentrations of 0.43, 0.85, 1.7, 3.4 and 6.8 ug/L. The top two doses represent gamma HBCD's water solubility (3.4 ug/L) and two times HBCD's water solubility (6.8 ug/L). A negative control and solvent control group were also included. Mean measured concentrations (LC/MS with heated nebulizer operated in the selective ion monitoring mode) were 0.25, 0.47, 0.83, 1.8 and 3.7 ug/L. This method was designed to monitor for all 3 HBCD diastereomers; however, the trace amounts of the alpha and beta diastereomers in the water samples were below the established limits of quantitation. Comparison of the chromatograms from study initiation through study termination showed that the relative distribution of the HBCD diastereomers remained constant during the definitive study, and the gamma diastereomer measured results were consistent throughout the study.

Hatching success, time to hatch, time for larvae to swim-up, and post-hatch growth and survival were evaluated during the 88-day test. Rainbow trout exposed to gamma HBCD at mean measured concentrations up to 3.7 ug/L (nominal concentration = 6.8 ug/L or twice gamma HBCD's water solubility) for a 27-day hatching period and 61 days post-hatch showed no effects on hatching success, time to swim-up, larval survival, fry survival or growth. Consequently, HBCD was not chronically toxic to rainbow trout at concentrations at or above its limit of solubility. The NOEC for this study was 3.7 ug/L or 6.8 ug/L nominal (twice gamma HBCD's water solubility). The low-effect-concentration (LOEC) and maximum acceptable toxicant concentration (MATC) could not be determined due to absence of toxicity, but were considered >3.7 ug/L or >6.8 ug/L nominal (> twice gamma HBCD's water solubility) (*Drottar et al. 2001. Hexabromocyclododecane (HBCD): An early life-stage toxicity test with the rainbow trout (Onchorhynchus mykiss). Project No.: 439A-112. Wildlife International, Ltd. Easton, MD*).

4.3..3.2 Flow Through Bioconcentration In Rainbow Trout (*Oncorhynchus mykiss*) (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD and GLP guidelines.

Nominal test concentrations were 0, 0.34, and 3.4 ug HBCD/L based on the gamma isomer. These doses are equivalent to gamma HBCD's water solubility and one tenth of its water solubility. Mean measured (LC/MS with heated nebulizer operated in the selected ion monitoring mode) test concentrations were 0, 0.18, and 1.8 ug HBCD/L. The length of the test was 70 days (35-day uptake, 35-day depuration). The steady bioconcentration factor (BCF) at a nominal concentration of 3.4 ug HBCD/L (1.8 ug HBCD/L measured) in whole fish was 8,974. This BCF was further defined as 4,650 in edible tissue and 12,866 in non-edible tissue. The isomer content in the fish was

approximately proportional to that of the test article. Steady state was not achieved at the nominal concentration of 0.34 ug HBCD/L due to an unexpected increase in tissue concentrations at day 35. The unexpected increase in tissue concentrations on day 35 may have been due to the variability in the measured water concentrations in this treatment group. The variability in turn is likely a function of the extremely low nominal concentration at this dose level (0.34 ug HBCD/L). Thus, the calculated BCF in the nominal 3.4 ug HBCD/L treatment group is considered a better estimate than that in the 0.34 ug HBCD/L treatment group (*Drottar K. and Krueger H. 2000. Hexabromocyclododecane (HBCD): Flow-through bioconcentration test with rainbow trout (Oncorhynchus mykiss). Project No.: 439A-111. Wildlife International, Ltd. Easton, MD*).

4.3.3.3 *Daphnia magna* Life Cycle (21 Day) (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD and GLP guidelines.

Nominal test concentrations were 0.85, 1.7, 3.4, 6.8 and 13.6 ug HBCD/L water; dose levels were based on gamma HBCD's water solubility, 3.4 ug/L. Measured test concentrations (LC/MS with negative ion atmospheric pressure ionization) were 0.87, 1.6, 3.1, 5.6 and 11 ug HBCD/L water (based on the gamma stereoisomer). No statistically significant effects on survival, reproduction or growth of *Daphnia magna* were seen at HBCD concentrations \leq 3.1 ug/L (measured). Thus, HBCD's no effect concentration (NOEC), based on survival, reproduction and growth, to daphnia magna for 21 days was equivalent to HBCD's water solubility. The measured NOEC in this study was 3.1 ug/L and corresponded to a nominal HBCD concentration of 3.4 ug/L, e.g. HBCD's water solubility. The lowest observed effect concentration (LOEC) and the maximum acceptable toxicant concentration (MATC) based on survival, growth and reproduction were greater than HBCD's water solubility. The LOEC, 5.6 ug/L, corresponded to nominal concentrations twice gamma HBCD's water solubility. The effect seen at this dose level was a reduction in length. Survival and reproduction at the 5.6 ug/L dose level were not affected. The MATC, 4.2 ug/L, was calculated as the mean of the NOEC and the LOEC. The MATC was greater than gamma HBCD's water solubility (*Drottar K. and Krueger H. 1998. Hexabromocyclododecane (HBCD): Flow-through life-cycle toxicity test with the cladocerna (Daphnia magna). Project No.: 439A-108. Wildlife International, Ltd. Easton, MD*).

4.3.3.4 Prolonged Sediment Study with *Hyalella azteca*, 5% TOC (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. The definitive study was performed according to current EPA, OECD and GLP guidelines.

Non-GLP exploratory range-finding studies were performed with three freshwater species associated with sediment: oligochaetes (*Lumbriculus variegatus*), chironomids (*Chironomus riparius*) and amphipods (*Hyalella azteca*). All three species were tested at 50, 100, 500, 1000 mg/Kg dry sediment in two different types sediments, one with a 2% organic carbon content and the other with a 5% organic carbon content. Based on the results of the range-finding studies, the amphipods were found to be the most sensitive species in both sediment types, with clear effects in the 500 mg/Kg treatment group. This reports the results of HBCD exposure in the 5% organic content sediment in *Hyalella azteca*. A similar study in 2% organic carbon was also performed.

Groups of amphipods were exposed to a geometric series of six test concentrations and a negative control for 28 days under flow-through test conditions. Eight replicate test compartments were maintained in each treatment and control group, with 10 amphipods in each test compartment, for a total of 80 amphipods per test concentration. Each test compartment contained a quantity of sediment and overlying water. Additional replicates were added in the control group, low and high treatment groups for analytical sampling of water and sediment. The “analytical” replicates sampled on Day 0 contained no amphipods, while amphipods were added at test initiation to the “analytical” replicates sampled on Day 7 and at test termination.

Nominal test concentrations were 31, 63, 125, 250, 500 and 1000 mg/Kg of sediment based on the dry weight of the sediment. The results of the study are based on the nominal test concentrations. Overlying water, pore water and sediment samples were collected and analyzed from the “analytical replicates” of the control group and the lowest and highest test concentrations. The collection and analysis were done approximately ten minutes after the addition of test organisms to the test system on Day 0, on Day 7 and at the end of the test. Results of the analyses were used to confirm the lowest and highest test concentrations.

Analysis of HBCD concentrations in the sediment, pore water and overlying water samples collected during test confirmed that the test article tended to remain in sediment and not move into the pore water or overlying water. Concentrations in sediment at the highest dose level ranged from 78.2 – 122% of nominal, while the lowest dose level ranged from below the limit of quantitation (12.5 mg/kg) to 197% of nominal. All overlying water samples contained no detectable HBCD. Pore water sample from the low concentration were also below the limit of quantitation, but those from the highest dose level, 1000 mg/kg sediment, had HBCD concentrations in the low ppm range. These values were all well above HBCD’s water solubility and were believed the result of small particles of HBCD being extracted out of the pore water, artificially inflating the reported values.

Observations of amphipods in individual replicates appeared normal, with some mortality in the 0, 31, 63, 125, 250 and 500 mg/kg groups. Fungal growth was noted in all replicates.

The mean number of amphipods in the negative control, 31, 63, 125, 250, 500 and 1000 mg/Kg treatment groups at test termination was 9.1, 8.6, 5.9, 6.1, 7.0, 8.5 and 9.1,

respectively. The mean numbers of amphipods in the 63, 125 and 250 mg/Kg treatment groups were found to be statistically different ($p \leq 0.05$) from the negative control group. Survival in the 31, 500 and 1000 mg/Kg treatment groups was similar to the control group and any differences were not statistically significant ($p > 0.05$). Since the percent reduction in the number of organisms present at test termination in comparison to the negative control group was less than 50% in all treatment groups, the 28-day EC50 value was estimated to be greater than 1000 mg/Kg of dry sediment, the highest concentration tested. The percent reduction from the control in the 63, 125, and 250 mg/Kg treatment groups was 35.2, 33.0, and 23.1%, respectively. The mortality in these groups was moderate and there were clearly no effects at the 500 and 1000 mg/Kg treatment levels. Therefore, the mortality observed in the middle test concentrations was not considered treatment related since there was no evidence of a concentration dependent response.

The average dry weight per amphipod in the negative control group was 0.19 mg. The average dry weight per amphipod in the 31, 63, 125, 250, 500 and 1000 mg/Kg treatment groups was 0.17, 0.26, 0.22, 0.20, 0.19 and 0.19 mg, respectively. The dry weights were not significantly different ($p > 0.05$) from the negative control weights, and any differences were not concentration-dependent. Therefore, there were no apparent effects on growth (dry weight) observed at test termination.

The 28-day EC50 value for amphipods (*Hyaella azteca*) exposed to hexabromocyclododecane in sediment was >1000 mg/Kg dry weight of sediment, the highest nominal concentration tested. Determination of the lowest-observed-effect-concentration (LOEC) and the no-observed-effect-concentration (NOEC) was based on an evaluation of the survival and growth (dry weight) data. The most sensitive parameter in this study was survival. Based on the results of this study, the LOEC was >1000 mg/Kg dry weight of sediment and the NOEC was 1000 mg/Kg dry weight of sediment. (Thomas J et al. 2003. *Hexabromocyclododecane (HBCD): A Prolonged Sediment Toxicity Test with Hyaella azteca Using Spiked Sediment with 5% Total Organic Carbon*. Project Number: 439A-120. Wildlife International, Ltd. Easton, MD.)

4.3.3.5 Prolonged Sediment Study with *Hyaella azteca*, 2% TOC (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. The definitive study was performed according to current EPA, OECD and GLP guidelines.

Non-GLP exploratory range-finding studies were performed with three freshwater species associated with sediment: oligochaetes (*Lumbriculus variegatus*), chironomids (*Chironomus riparius*) and amphipods (*Hyaella azteca*). All three species were tested at 50, 100, 500, 1000 mg/Kg dry sediment in two different types sediments, one with a 2% organic carbon content and the other with a 5% organic carbon content. Based on the results of the range-finding studies, the amphipods were found to be the most sensitive species in both sediment types, with clear effects in the 500 mg/Kg treatment group. This

reports the results of HBCD exposure in the 5% organic content sediment in *Hyalella azteca*. A similar study in 5% organic carbon was also been performed.

Groups of amphipods were exposed to a geometric series of six test concentrations and a negative control for 28 days under flow-through test conditions. Eight replicate test compartments were maintained in each treatment and control group, with 10 amphipods in each test compartment, for a total of 80 amphipods per test concentration. Each test compartment contained a quantity of sediment and overlying water. Additional replicates were added in the control group, low and high treatment groups for analytical sampling of water and sediment. The “analytical” replicates sampled on Day 0 contained no amphipods, while amphipods were added at test initiation to the “analytical” replicates sampled on Day 7 and at test termination.

Nominal test concentrations were 31, 63, 125, 250, 500 and 1000 mg/Kg of sediment based on the dry weight of the sediment. The results of the study are based on the nominal test concentrations. Overlying water, pore water and sediment samples were collected and analyzed from the “analytical replicates” of the control group and the lowest and highest test concentrations. The collection and analysis were done approximately ten minutes after the addition of test organisms to the test system on Day 0, on Day 7 and at the end of the test. Results of the analyses were used to confirm the lowest and highest test concentrations.

Analysis of HBCD concentrations in the sediment, pore water and overlying water samples collected during test confirmed that the test article tended to remain in sediment and not move into the pore water or overlying water. Concentrations in sediment at the highest dose level ranged from 82.8 – 115% of nominal, while the lowest dose level ranged from 49.5 to 125% of nominal. All overlying water samples contained no detectable HBCD. Pore water sample from the low concentration were also below the limit of quantitation, but those from the highest dose level, 1000 mg/kg sediment, had HBCD concentrations in the low ppm range. These values were all well above HBCD’s water solubility and were believed the result of small particles of HBCD being extracted out of the pore water, artificially inflating the reported values.

Observations of amphipods in individual replicates appeared normal, with some mortality in the 0, 31 and 1000 mg/kg groups. Fungal growth was noted in all replicates.

The mean number of amphipods in the 0, 31, 63, 125, 250, 500 and 1000 mg/kg groups at test termination was 7.4, 5.9, 7.8, 5.4, 6.9, 7.3, and 5.8, respectively. No statistical difference between treated and control groups were detected. The average dry weight per amphipod in the 31, 63, 125, 250, 500 and 1000 mg/kg treatment groups was 0.10, 0.13, 0.14, 0.12, 0.17 and 0.14, respectively, and were not significantly different from control ($p>0.05$).

Based on survival and growth (dry weight) data, the 28-day EC50 was > 1000 mg/kg of dry sediment, the highest concentration tested. The LOEC was > 1000 mg/kg and the NOEC was 1000 mg/kg dry weight of sediment. (Thomas S, Krueger H and Kendall T.

2003. Draft Report. Hexabromocyclododecane (HBCD): A Prolonged Sediment Toxicity Test with *Hyalella azteca* Using Spiked Sediment with 2% Total Organic Carbon. Project Number: 439A-119B. Wildlife International, Ltd. Easton, MD.)

4.3.4 Terrestrial Organisms

4.3.4.1 Earthworm (*Eisenia fetida*) Survival and Reproduction

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD and GLP guidelines.

The artificial soil used in this study was characterized as sandy loam with an 80% sand, 8% silt, and 12% clay content. Nominal test concentrations were 0, 78.5, 157, 313, 625, 1,250, 2,500 and 5,000 mg HBCD/kg of dry soil. Mean measured concentrations at day 28 were <1.28 (Control), 61.2, 145, 244, 578, 1,150, 2,180, and 4,190 mg HBCD/kg of dry soil. Mean measured concentrations at day 56 were 56 Days: <1.35 (Control), 51.5, 128, 235, 543, 1,070, 2,020, and 3,990 mg HBCD/kg of dry soil. Measured tissue concentrations at day 28 were <0.200 (control), 3.40, 7.32, 16.8, 15.3, 53.0, 71.2, and 150 µg HBCD per gram of tissue.

After 28 days of exposure to HBCD, percent mortality of the adult worms was 0, 5, 0, 0, 0, 0, 3, and 0% in the 0, 61.2, 145, 244, 578, 1,150, 2,180, and 4,190 mg HBCD/kg groups, respectively. All of the live earthworms were normal in appearance and behavior. No abnormal burrowing or avoidance behaviors were recorded during the first 60 minutes of testing.

The control worms gained an average of 0.418 g per replicate or 10% in replicate mass during the 28 days of adult worm exposure. The mean replicate weight gain of the surviving treatment animals ranged from 0.018 to 0.808 grams. These gains represented average increases of 0.4 to 19% in replicate animal mass over the initial 28 days of exposure.

The average reproduction in the control replicates was 72 juveniles per replicate. The coefficient of variation for the control data was 16%. The average reproduction was 61, 60, 49, 31, 26, 26, and 30 juveniles per replicate for treatment levels 51.5, 128, 235, 543, 1,070, 2,020, and 3,990 mg HBCD/kg, respectively. A statistically significant ($p \leq 0.05$) reduction in reproductive output occurred at treatment levels ≥ 235 mg HBCD/kg.

Using HBCD's estimated soil K_{oc} (Table 1) and the formula provided in the European Union's Technical Guidance Document, HBCD's calculated BCF in earthworms is:

$$BCF_{\text{earthworm}} = \frac{C_{\text{earthworm}}}{C_{\text{soil}}} = K_{\text{earthworm-porewater}} \times \frac{RHO_{\text{soil}} \times 10+3}{K_{\text{soil-water}}}$$

$$(0.15 \text{ mg/kg})(1.25 \times 10+5)/4,190 \text{ mg/kg dry soil} = 4.5.$$

Thus, HBCD did not bioconcentrate in the earthworm.

The 28-Day EC₅₀ (survival) was >4,190 mg/kg. The 28-Day NOEC (survival) was 4,190 mg/kg. The 56-Day EC₅₀ (reproduction) was 771 mg/kg with 95% confidence limits of 225 to 4,900 mg/kg. The 56-Day NOEC (reproduction) was 128 mg/kg. (Aufterheide *et al.* 2002. *Effect of Hexabromocyclododecane on the Survival and Reproduction of the Earthworm, Eisenia fetida*. ABC Study No. 47222. ABC Laboratories, Inc., Columbia, Missouri; Wildlife International, Inc., Easton, MD.)

4.3.4.2 Terrestrial Plants

The purpose of the study was to determine the effects of Hexabromocyclododecane (HBCD) on the seedling emergence and growth of six species of non-target plants. The experimental design for this study consisted of a negative control and five treatment groups. Each group had four replicate pots with ten seeds planted in each pot. Application of test concentrations of HBCD was made by soil incorporation to each treatment group prior to the planting of seeds. The nominal test substance concentrations were 0, 40, 105, 276, 725, 1,904, and 5,000 mg of HBCD per kilogram of dry soil (mg HBCD/kg). The mean measured test levels were 0 (Negative Control), 31.3, 97.8, 297, 764, 2,230, and 6,200 mg HBCD/kg dry soil.

Seeds were impartially assigned to pre-labeled growth pots on the day of test initiation. The replicate pots were placed in a randomized block design on a greenhouse table after planting. Observations of emergence were made on Days 7, 14, and 21. A general assessment of seedling condition was made on Day 7, while observations of height, shoot dry weight, and assignment of plant condition scores were made only on Day 21.

There were no apparent effects on any endpoint for any of the six species tested. Statistical analyses indicated that there were no significant differences (Dunnett's test, $p > 0.05$) between the control and treatment group mean emergence, survival, height, or weight for corn, cucumber, ryegrass, soybean and tomato. On day 21, onion showed a statistically significant difference (Dunnett's test, $p < 0.05$) between the control and the 276 mg HBCD/kg treatment group mean survival. This significant difference was not considered dose-responsive, and not attributed to treatment, as no statistical differences were noted at higher concentrations tested (Dunnett's test, $p > 0.05$). There were no statistically significant differences (Dunnett's test, $p > 0.05$) between the control and treatment group mean emergence, height or weight for onion. Additionally, there were no signs of treatment-related phytotoxicity observed on seedlings of any species at any test concentration.

No effects from soil incorporation of HBCD were observed on seedling emergence, survival, or growth for any of the six plant species tested. Therefore, the NOEC for emergence and growth of all seedlings in this study was determined to be 5,000 mg of HBCD/kg, which was the highest nominal soil concentration tested. (Porch *et al.* 2002. *Hexabromocyclododecane (HBCD): A Toxicity Test to Determine the Effects of the Test Substance on Seedling Emergence of Six Species of Plants*. Project Number: 439-103. Wildlife International, Ltd. Easton, MD.)

4.4 MAMMALIAN TOXICOLOGY DATA

HBCD was not acutely toxic to rats on oral or dermal exposure. In repeated dose studies in rats (28 and 90-day studies), HBCD's no adverse effect level (NOAEL) was 1,000 mg/kg/day. HBCD did not induce developmental effects in the rat (NOAEL = 1,000 mg/kg/d). No evidence of carcinogenicity was found in an 18 month mouse study. HBCD did not induce mutations in the Ames, *in vitro* chromosome aberration, and *in vivo* mouse micronucleus tests. HBCD was negative for contact sensitization in the mouse local lymph node assay.

4.4.1 Acute Mammalian Toxicology Data

HBCD was not acutely toxic to rats or rabbits during oral, dermal or inhalation exposure. The rat oral LD50 was >10 g/kg. The rabbit dermal LD50 was >8 g/kg. The rat inhalation LC50 was > 200 mg/L. HBCD was not irritating to the skin or eye when tested in rabbits. (Lewis C. 1978. *Experiment Reference No. 78385-2 and 78385-1. Consumer Product Testing, Fairfield, NJ*).

4.4.2 Repeated Dose Toxicology Data

In repeated dose studies in rats, HBCD's no adverse effect level was at or near 1,000 mg/kg/day. Two 28-day studies and two 90-day studies have been performed.

4.4.2.1 Rat 28-Day Subchronic (BFRIP)

This study was conducted according to OECD and GLP guidelines. The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc.

HBCD, in the vehicle corn oil, was administered orally by gavage to three groups of Sprague-Dawley Crl: CD BR rats for a period of 28 consecutive days. Dose levels were 125 (low), 350 (mid), or 1,000 (high) mg/kg/day, administered at dosage volume of 5 ml/kg. The test groups consisted of 6 males and 6 females in the 125 and 350 mg/kg/day groups and 12 males and 12 females in the 1000 mg/kg/day group. A concurrent control group comprised of 12 males and 12 females received the vehicle, corn oil, for 28 consecutive days at a dosage volume of 5 ml/kg. 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/day groups remained on-test untreated for a 14-day recovery period. At the end of the recovery period, all animals were sacrificed and necropsied.

Animals were observed twice daily for mortality and moribundity. Clinical signs were recorded daily. Body weight and food consumption were measured weekly. Functional observational battery and motor activity evaluations were performed during weeks -1 (pretest), 3, and 5 (recovery). Samples for hematology and serum chemistry evaluations were collected at the primary (28 day) and recovery (42 day) sacrifices. Complete necropsies were performed on all rats. The brain, liver, kidney, heart, spleen, testes and

epididymus or ovaries, adrenal glands, and thymus from all animals were weighed at each sacrifice. Approximately 40 tissues were collected and preserved at each necropsy from all animals. The following tissues were examined microscopically from the control and high dose animals: liver, kidney, heart, spleen, testes (males), prostate (males), seminal vesicles (males), epididymus (males), ovaries (females), adrenal glands, thymus, bone with marrow (sternebra), brain, stomach, cecum, duodenum, ileum, jejunum, lymph node, peripheral nerve (sciatic), spinal cord, lung, trachea, uterus (females), urinary bladder, and all gross lesions. The lungs, liver, kidneys, stomach, thyroid, gross lesions and target organs were examined in all dose levels.

Survival was not affected by administration of the test article. All animals survived to the scheduled sacrifice. Clinical signs observed during the study were nonspecific, low in incidence, non-dose-related and not considered related to test article.

Body weights, weight gain and food consumption of treated animals were compared statistically by sex and treatment day to their respective control groups ($p \leq 0.05$ or 0.01) and were not affected by treatment. No statistically significant differences in body weight between control and treated animals were detected with the exception of an increase in mean female body weight in the 350 mg/kg/day group during week 2 of treatment. Mean female body weight at that time point was 196 g versus 179 in the control group. No statistically significant differences in body weight gain between control and treated animals were detected with the exception of a decrease in mean male body weight gain in the 1,000 mg/kg/day recovery group during week 1 of recovery. Mean male body weight gain at that time point was 21 g versus 31 in the control group; mean male body weight was not statistically different from the control mean. No statistically significant differences in food consumption between control and treated animals were detected with the exception of an increase in mean female food consumption in the 350 mg/kg/day group during weeks -1, 1, and 2 of treatment. Mean female food consumption at that those time points were 18, 17 and 17 g versus 16, 15 and 15 g in the control group, respectively.

Functional observation battery and motor activity results from treated animals were compared statistically by sex and treatment day to their respective control groups ($p \leq 0.05$). These parameters were not affected by treatment with the test article. No statistically significant differences were observed between treated and control animals at any time point.

No statistically significant differences between treated and control animals were found for hematology parameters with the exception of an increase in the mean activated partial thromboplastin time in the 1000 mg/kg/day males on week 4 and a decrease in the mean prothrombin time in the 1000 mg/kg/day females on week 4. These statistical differences were not of toxicological significance.

No toxicologically significant effects on serum chemistry values related to test article administration were observed at the 28-day primary and 42-day recovery sacrifice. Scattered instances of statistically significant differences between treated and control

animals were detected for some serum chemistry parameters at the 28-day primary sacrifice. These scattered statistical differences were not considered toxicologically significant because the statistical differences occurred: in the absence of a dose response, in the absence of the accompanying clinical chemistry changes expected, in the opposite direction from what occurs in a toxic state, in a direction which is without physiologic significance, or due to potential interference with the laboratory method. No statistically significant differences in serum chemistry parameters were detected between groups at the 42-day recovery sacrifice.

No gross lesions that could be attributed to the test article were detected at either necropsy. Gross lesions were nonspecific, low in incidence, non-dose-related and considered incidental.

No microscopic lesions that could be attributed to the test article were detected on histopathologic exam. Microscopic changes were nonspecific, low in incidence, non-dose-related and considered incidental.

No statistical significant differences in organ weight or organ to body weight ratios were detected between control and treated animals with one exception. Absolute liver weights were statistically significantly increased with respect to control means at the 28-day sacrifice in males in the high dose and females in the mid and high dose. Liver to body weight ratios in mid and high dose males and low, mid and high dose females were statistically significantly increased at the 28-day sacrifice. At the recovery sacrifice, male absolute and liver to body weight ratio were statistically comparable to the control mean whereas female absolute liver weights and liver to body weight ratio were statistically significantly increased with respect to control mean. The difference in absolute liver weight between control and treated females was less pronounced at the end of the recovery period, indicating the increase in liver weight was reversible in females as well as males. In the absence of test article related histopathologic and serum chemistry changes, increases in liver weight are considered an adaptive, rather than a toxic response, are not uncommon in the rat, and are most likely the result of microsomal induction.

In conclusion, no systemic toxicity was observed at any dose level. Based on the results of this study, the NOAEL (No Observed Adverse Effect Level) of HBCD administered orally to male and female rats for 28 consecutive days was 1,000 mg/kg/day (*Chengelis C. 1996 A 28-day repeated dose oral toxicity study of HBCD in rats. Study No. WIL-186004. WIL Research Laboratories, Inc. Ashland, OH*).

4.4.2.2 Rat 90-Day Subchronic (BFRIP)

This study was conducted according to OECD and GLP guidelines. The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc.

The test article, a composite of three lots of commercial hexabromocyclododecane (HBCD), was administered by oral gavage in corn oil once daily to four groups of Crl:CD(SD)IGS BR rats (n=15/sex/group) at dose levels of 0 (control), 100 (low), 300 (mid) and 1000 (high) mg/kg/day seven days per week for 90 days. The dosage volume was 5 ml/kg. The control animals received the vehicle, corn oil, only. At the end of the 90-day treatment period, 10 animals/sex/group were euthanized and necropsied. The remaining rats continued on test untreated for a 28-day recovery period prior to necropsy.

In addition to the main toxicology groups, two satellite groups of 20 animals/sex/group were treated concurrently in an identical manner at dose levels of 0 or 1000 mg HBCD/kg/day for up to 90 days. Body weights were recorded weekly. Two animals/sex/group were euthanized on study days 2, 6, 9, 13, 20, 27, 55, 89, 104 and 118, and blood and body fat (mesenteric and/or omental) were collected. The body fat was analyzed for HBCD content.

Animals in the main toxicology groups were observed twice daily throughout the study for mortality and morbidity. Body weights and food consumption were measured weekly. Blood was collected at study weeks 3 (n=5/sex/group), 13 (n=10/sex/group) and 17 (n=5/sex/group) for hematology, serum chemistry and hormone (T₃, T₄ and TSH) measurements. Urine was collected prior to each necropsy, at study weeks 13 and 17, for urinalysis. Ocular examinations were performed prior to study initiation and during study weeks 12 and 15. Functional Observational Battery and Locomotor Activity evaluations were performed on 5 animals/sex/group prior to study initiation, during the last week of test article administration (study week 13), and during the recovery period. An examination of vaginal cytology (for estrus cycle determinations) was performed on study days 69-90. At each necropsy, sperm motility/viability, morphology, and number were assessed. Complete necropsies were performed on all animals. Approximately 40 organs or tissues/animal were collected and preserved. The adrenals, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus, thyroids with parathyroids, and uterus with cervix were weighed. Paraffin sections of tissues stained with hematoxylin and eosin from the control and 1000 mg/kg/day dose groups and the liver, lungs and thyroid glands in the 100 and 300 mg/kg/day doses, and gross lesions from all animals were examined under the light microscope. Livers from five randomly chosen animals/sex from the control and 1000 mg/kg/day dose groups were examined microscopically using Oil Red O or periodic acid Schiff's (PAS) reagent for evidence of lipid accumulation or glycogen accumulation/depletion, respectively. Statistical comparisons by sex and treatment day were made between the control and treated animals where indicated (p<0.05).

No test article-related effect on mortality occurred. Clinical signs were non-specific, low in incidence, non-dose-related and not related to test article administration. No test article-related changes occurred in body weight, food consumption, Functional Observational Battery or Locomotor Activity. No test article-related effects on hematologic parameters were noted. No test article-related ocular lesions were detected at the ophthalmic exams. No test article-related changes were noted on the estrus cycle as determined by vaginal cytology, or on sperm motility/viability, morphology, and

number. Instances of statistically significant differences between control and some treatment groups were detected at study week 13 in the clinical chemistry data, hormone data, organ weight data and histology findings. They were considered secondary to hepatic enzyme induction or were otherwise not considered adverse effects of treatment as discussed further below.

Statistically significant ($p < 0.05$) test article-related serum clinical chemistry changes at week 13 include an increase in albumin (all dose levels for males), total protein (all dose levels for females and 1000 mg/kg/day for males), globulin (300 and 1000 mg/kg/day for females), and chloride (all doses for both sexes). In addition, increased gamma glutamyltransferase levels were noted in the 1000 mg/kg/day group ($p < 0.05$). Thyroxine (T_4) levels were decreased at study week 13 compared to the control mean in all male dose groups and the 300 and 1000 mg/kg/day dose females ($p < 0.05$). There were no corresponding statistical effects on T_3 and TSH.

While potentially test article-related, the changes in serum chemistry parameters were not of sufficient magnitude to be adverse, occurred in otherwise clinically normal animals, tended to be within or close to historical control values, and were not present at the end of the recovery period. The increased serum albumin and gamma glutamyltransferase levels were considered related to the increased in liver weight in treated animals that was believed due to enzyme induction. Hepatic GGT is inducible in the rat (*Teschke et al 1983. Gut. 24(7):625-30; Chandar and Nagarajan 1984. Biochem Int. Jan;8(1):41-8*), and this induction has been shown to increase serum GGT levels in the rat (*Goldberg 1980. Crit Rev Clin Lab Sci. 12(1):1-58; Teschke et al. 1983. Gut 24(7):625-30; Nishmura and Teschke 1982. Biochem Pharmacol. Feb 1;31(3):377-81; Satoh et al. 1982. J Pharmacol Exp Ther. Jun ;221(3):795-800*). The increase in serum chloride was considered secondary to be presence of free bromide in the test article preparation that interfered with the chloride determination methodology. The decrease in T_4 , which was also reversible, was considered related to the increased liver weights in that induction of hepatic UDPG is known to enhance the elimination of T_4 . The rat is particularly sensitive to hepatic enzyme induction.

The incidence of observations noted at gross necropsy was low and was not accompanied by evidence of frank organ damage. On histopathologic examination, mild findings were detected in both the control and treated groups. Potential test article-related histologic changes were identified in the liver and thyroid glands, but these were not considered to be indicative of frank toxicity. The liver changes in male rats at the 90-day necropsy (Study Week 13) were characterized as minimal hepatocellular vacuolation and occurred in 10% of control males and ~50% of the males at 100, 300 and 1000 mg/kg/day. Thus, there was no increase in incidence with dose. Minimal hepatocellular vacuolation was also detected in females in the control and treated groups without a clear dose response (3 to 4/10 animals per group). Mild and moderate vacuolation was detected in females only in the 300 (1/10) and 1000 mg/kg/day (2/10) dose groups. Minimal to mild hepatocellular hypertrophy was also detected only in the 1000 mg/kg/day group (5/10) females. Minimal thyroid follicular cell hypertrophy was detected 1/10, 1/10, 5/10 and 7/10 males in the control, 100, 300 and 1000 mg/kg/day groups, respectively and in 4/10

and 3/10 females in the 300 and 1000 mg/kg/day groups respectively. In addition, mild thyroid follicular hypertrophy was detected in 4/10 females in the 1000 mg/kg/day group.

The histologic changes in the liver were accompanied by an increase in liver weight. In contrast there were no statistically significant changes in thyroid weight (absolute, relative to body weight and relative to brain weight). At study week 13, mean liver weights in all dose levels of both sexes (absolute, relative to body weight and relative to brain weight) were increased compared to the male and female control means ($p < 0.05$).

The changes seen in this study, an increase in liver weight, the lack of adverse histologic effects in the liver and the apparent normal functioning of the liver, are consistent with enzyme induction (*Amacher et al. 1998. Food Chem. Toxicol. 36:831-830*), and hepatic enzyme induction is an adaptive, and not an adverse, effect (*Williams and Iatropoulos 2002. Toxicol Pathol. Jan-Feb;30(1):41-53*). Hepatocellular vacuolation and hypertrophy, seen in this study, often accompany the increased liver weight caused by liver enzyme induction, and were reversible. At week 17, the liver changes (weight and histology) had at least partially, if not fully, resolved in all treated groups without delayed or long-term toxic effects.

Limited pharmacokinetic studies indicate HBCD is extensively metabolized prior to excretion in the feces and urine. The results of the fat analysis in this study indicate that mammalian system handles the 3 HBCD stereoisomers differently, and may be less efficient at eliminating one stereoisomer over another. (The relative isomer concentrations in adipose tissue at all time points were $\alpha \gg \gamma > \beta$ in contrast to the test article composition of $\gamma \gg \alpha > \beta$.) Thus, it should not be unexpected that hepatic enzyme induction occurs with exposure to substantial doses over a significant period of time, as was the case in the 90-day study.

The histologic 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. It was not readily apparent that these minimal changes were an effect of treatment, and in any event appeared reversible. The follicular cell hypertrophy may have been related to serum T₄ levels. Follicular cell hypertrophy is the normal physiological response to reduced to serum T₄ levels and is the typical adaptive response of a healthy normally functioning organism acting to maintain serum T₄ levels in the normal range.

The mean prostate weight was increased in the 1000 mg/kg/day group males at the primary necropsy. This was not considered to be of toxicological significance since the increase did not persist to the recovery period, there were no correlating histologic findings and no change in seminal quality.

HBCD was detected in the adipose tissue of male and female rats treated with 1000 mg/kg/day for up to 90 days. Isomer-specific analysis showed that the relative isomer concentrations in adipose tissue at all time points were $\alpha \gg \gamma > \beta$ which is in contrast to the test article composition ($\gamma \gg \alpha > \beta$). 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.

All the test article-related changes at 100 and 300 mg/kg/day were mild, reversible, generally secondary to hepatic enzyme induction (which is an adaptive not a toxic change) and without effect on the clinical condition of the animals. The findings observed at 1000 mg/kg/day (increased serum gamma glutamyltransferase, increased liver and prostate weights), were also reversible, not associated with specific target organ damage or diminished function and were, therefore, of limited, if any, toxicologic significance. On this basis, the no-observed-adverse-effect level (NOAEL) of HBCD administered to Crl:CD[®](SD)IGS BR rats by gavage in corn oil for 90 days is 1000 mg/kg/day (*Chengelis C. An Oral (Gavage) 90 Day Toxicity Study of HBCD in Rats. Study No. WIL-186012. WIL Research Laboratories, Inc., Ashland, Ohio. 2001*).

4.4.2.3 Rat 28-Day Subchronic (BASF)

HBCD (“Hexabromid S”) was tested in Sprague-Dawley rats (10/sex/group) at doses of 0, 1, 2.5 and 5% of the diet for 28 days. Doses calculated from the actual body weights and food consumption in this study are 0, 940, 2410, and 4820 mg/kg body weight/day.

No clinical signs related to treatment were observed at the 1% dose level. Body weights at the 1 and 2.5% dose levels were comparable to the controls. Liver weights (absolute and relative to body weight) were increased at all dose levels, but no microscopic pathology was detected. Thyroid hyperplasia was observed in some animals at all doses, and “very slight numerical development of the follicles and ripening follicles in the ovaries of females” at the high dose (4820 mg/kg/d) was reported. No changes in any other organ related to treatment and no changes in clinical chemistry tests were detected.

The report concluded that “The increased liver weight must be attributed to hyperactivity; hypermetabolism as a result of increased thyroid activity appears probable in view of the observations of the thyroid”. Therefore, the increased liver weights were not pathologic: there were no microscopic lesions detected on histopathology and no change in clinical chemistry values (*Zeller H and Kirsch P (1969) Hexabromocyclododecane: 28-day feeding trials with rats. BASF Unpublished Laboratory Report*).

Recent work on the relationship of liver weight, microsomal enzyme induction, and histological change in rat toxicology studies has been published (*Amacher et al, Food and Chemical Toxicology, 36, 831-839, 1998*). Amacher et al. concluded “The preponderance of data collected in these 11 studies indicates that microsomal enzyme induction was not accompanied by evidence of chemically-induced liver injury. We conclude that in the rat, both hepatomegaly and microsomal enzyme induction are benign and adaptive changes in response to certain chemicals that stimulate the hepatic drug metabolizing enzyme system.”

4.4.2.4 Rat 90-Day Subchronic (BASF)

HBCD (“Hexabromid S”) was tested in Sprague-Dawley rats at doses of 0, 0.16, 0.32, 0.64 and 1.28% of the diet for 90 days. Doses calculated on the actual body weights and food consumption in this study reveals: 0, 120, 240, 470 and 950 mg/kg body weight/day.

Doses up to 0.64% (470 mg/kg/d) produced no adverse clinical signs, no change in body weight, and no change in clinical chemistry results. An increase in the relative liver to body weight ratio was found, and was accompanied by fatty accumulation but no other histologically discernible changes were detected in the liver. Further, no histological changes were found in any other organ. The original report stated that in the “absence of detectable clinico-chemical disturbances or histological changes of the vital organs, it was concluded that the increased liver weight and the fat deposits, both of which were largely reversible when administration of Hexabromid S was stopped, were the result of a temporary increase in the activity of the liver.” Thus, no adverse effect was produced at the highest dose tested, 1.28% of the diet (*Zeller H and Kirsch P (1970) Hexabromocyclododecane: 90-day feeding trials with rats. BASF Unpublished Laboratory Report*).

4.4.3 Genetic Toxicity – Mutation

HBCD did not induce genetic toxicity when tested in the Ames, *in vivo* mouse micronucleus, or *in vitro* chromosome aberration tests.

4.4.3.1 Ames Salmonella

HBCD has been tested for mutagenicity in the Ames Salmonella microsomal assay, both with and without metabolic activation, in multiple tests. All results were negative (*Ogaswara S and Hanafusa T. (1993) Report on mutagenicity test on Pyroguard SR-103 using microorganisms; Baskin A and Phillips, B. (1977) Mutagenicity of two lots of FM-100, Lot 53 and residue of Lot 3322 in the absence and presence of metabolic activation. Industrial Biotest Laboratories, Sponsored by Velsicol Chemical Corporation; Anonymous. (1979) Mutagenicity test of GLS-S6-41A. Gulf South Research Institute, Sponsored by Ethyl Corporation; US Environmental Protection Agency (1990) Ames metabolic activation test to assess the potential mutagenic effect of Compound No. 49. Letter from BASF. EPA/OTS Doc #86-900000385; Simmons V., Poole, D., Newell, G., and Skinner, W. (1976) In vitro microbiological mutagenicity studies for four CIBA-GEIGY Corporation compounds. SRI Project LSC-5702.*).

4.4.3.2 In Vivo Mouse Micronucleus (BASF)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current OECD guidelines and Good Laboratory Practices.

HBCD dose levels administered intraperitoneally to male mice were 0, 500, 1,000 or 2,000 mg/kg body weight. The negative control animals were administered the vehicle, DMSO.

Cyclophosphamide and vincristine were used as positive controls and responded as expected. HBCD-treatment did not increase in number of polychromatic erythrocytes containing either small or large micronuclei. Micronuclei formation in HBCD-treated mice was within the same range as that of the concurrent negative control and within the range of historical control data. No evidence of chromosome damaging (clastogenic) effects was observed. There was no indication of any impairment of chromosome distribution in the course of mitosis. HBCD was clearly negative for clastogenicity and the ability to induce spindle poison effects in this mouse micronucleus test (*Engelhardt, G and Hoffmann, H. (2000) Laboratory Project Identification: 26M0100/004018, Experimental Toxicology and Ecology, BASF Aktiengesellschaft, Ludwigshafen, Germany*).

4.4.3.3 *In Vitro* Iatrogenic Recombination

The Sp5 and SPD8 cell lines were developed by the paper's authors. The clones used in this study exhibit a spontaneous partial duplication of the hprt gene, resulting in a non-functional hgprt protein. These mutants revert spontaneously to a functional hprt gene phenotype by recombination with a frequency of 1×10^5 reversions/cell generation. This reversion frequency is said to increase by exposure to chemical or physical agents. Treatment with the test substance was for 24 hr at 37 degrees C. HBCD was tested *in vitro* in hamster cells (Sp5/V79 and SPD8) in a recombination assay at five doses between 2 and 20 ug/ml plus a control. In the SPD8 cells, HBCD concentrations of 0, 3, 6, 10, 15, and 20 ug/ml resulted in a reversion frequency of 1.0, 0.7, 0.8, 0.9, 1.4, and 1.9, respectively. Cytotoxicity was observed at the 20 ug/ml dose. In the Sp5 cells, HBCD concentrations of 0, 2, 5, 10, 15, 20 ug/ml resulted in a reversion frequency of 1.0, 1.0, 0.8, 1.1, 1.4 and 2.2, respectively. Cytotoxicity was not observed. The reversion frequency at the 20 ug/ml dose for the Sp5 and SPD8 cells was statistically different from the control (Student's t test, $p < 0.05$). Treatment with HBCD resulted in an ~ maximal 2-fold increase in revertant frequency. (*Helleday et al. Brominated flame retardants induce intragenic recombination in mammalian cells. Mutation Research 439 (1999) 137-147*).

This is a non-standard genetic toxicity test, and its reliability and predictive ability is unknown. This is not a test used by regulatory agencies to assess genotoxicity potential.

4.4.3.4 Genetic Toxicity – *In Vitro* Chromosome Aberration (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. This study was performed according to current EPA, OECD and GLP guidelines.

HBCD was tested in the *in vitro* mammalian cytogenetic test using human peripheral blood lymphocytes both in the absence and presence of metabolic activation. The assay was performed in two 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 assay.

Dimethylsulfoxide was used as a solvent. In the initial assay, the maximum dose tested was 2,500 ug/ml. Dose levels greater than 2,500 ug/ml were insoluble in treatment medium. Visible precipitate was observed in treatment medium at 750 and 2,500 ug/ml and was soluble but cloudy at dose levels of 75 and 250 ug/ml. The test article was soluble in treatment medium at all other doses tested. In the non-activated portion of the initial assay cells were exposed to the test article continuously for 20 hours; in the S9-activated portion of the initial chromosome aberration assay, cells were exposed to the test article for 4 hrs. Metaphase cells were collected at 20 hrs after initiation of treatment. Dose levels of 2,500 ug/ml in the non-activate study and 750 and 2,500 ug/ml in the S9-activated study were not analyzed for chromosome aberrations due to complete mitotic inhibition. Toxicity (mitotic inhibition) of ~56% was observed at the highest dose level (750 ug/ml) evaluated for chromosome aberrations, in the non-activated study. In the S9-activated study, 13% toxicity was observed at the highest dose level (250 ug/ml) evaluated for chromosome aberrations. No statistically significant increases in chromosome aberrations were observed in either the non-activated or S9-activated test systems relative to the solvent control group regardless of dose level.

Based on the results of the initial assay, an independent repeat chromosome aberration assay was conducted in the absence and presence of an Arochlor-induced S9 metabolic activation system at dose levels of 10, 19, 38, 75, 150, 300 and 600 ug/ml. The test article was soluble but cloudy at 75 ug/ml and was workable in treatment medium at dose levels 150 ug/ml and higher. The test article was soluble in treatment medium at all other concentrations tested. In the independent repeat assay, cells were exposed to the test article continuously for 20 or 44 hr in the non-activated test system and for 4 hours in the S9-activated test system. Metaphase cells were collected for microscopic evaluation in both the non-activated and S9-activated studies at 20 and 44 hrs after initiation of treatment. Toxicity, measured by mitotic inhibition, was ~55% and 94% at the 20 and 44 hr harvests, respectively, at the highest dose levels (600 and 300 ug/ml) evaluated for chromosome aberrations in the nonactivated studies. In the S9-activated studies, toxicity was approximately 71% and 69% at the 20 and 44 hr harvests, respectively, at the highest dose levels (600 and 300 ug/ml) evaluated for chromosome aberrations. The 600 ug/ml dose level in the non-activated 44 hr harvest and in the S9-activated 20 hr harvest was not analyzed for chromosome aberrations due to an insufficient number of scorable metaphase cells. No statistically significant increases in structural chromosome aberrations were observed in either the non-activated or S9-activated studies, regardless of dose level or harvest time. No statistically significant increases in numerical chromosome aberrations were observed in either the non-activated or S9-activated studies at the 44 hr harvest time, regardless of dose level. HBCD was negative for the induction of structural and numerical chromosome aberrations in human peripheral blood lymphocytes (*Gudi, R. and Schadly, E. 1996. Laboratory Study Number G96AO61.342. Microbiological Associates, Inc., Rockville, MD*).

4.4.4 Developmental Toxicity Data

Two developmental toxicity studies at doses up to 1,000 mg/kg/d have been performed in the rat. Neither was positive for the induction of maternal or fetal toxicity or developmental effects.

4.4.4.1 Rat Prenatal Developmental Toxicity (BFRIP)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. The study was performed according to EPA, OECD and GLP guidelines. This study was required by KEMI (without consultation of the EU Technical Meeting) because KEMI decided the existing study in the literature (*Murai et al.*) was insufficient.

HBCD was administered in corn oil by gavage to 25 presumed pregnant Crl:CD(SD)IGS Br rats/group once daily from gestation days 6-19 at doses of 0, 250, 500 or 1,000 mg/kg/day. Control animals received corn oil only. Female rats were mated in-house and were treated daily on gestation days 6-19 with HBCD via gavage at dose levels of 0 (vehicle control), 250, 500 or 1000 mg/kg/day at a constant volume of 5 ml/kg. Individual doses were based on the most recent body weight. The day on which evidence of mating was observed was considered day 0 of gestation. Dams were observed daily and maternal body weight and food consumption measured at appropriate intervals. Females were euthanized on day 20 of gestation and necropsied. Gravid uterine and liver weights were recorded. Litters were delivered by cesarean section. The total number of corpora lutea, total number of implantations, early and late resorptions, number and location of all fetuses, and the sex and individual weights of fetuses were recorded. All fetuses were examined grossly. Approximately one-half of the fetuses in each litter were stained with Alizarin Red S and Alcian Blue and evaluated for skeletal/cartilaginous malformations and ossification variations. The maternal day 20 gestation examinations and cesarean sections, and subsequent fetal evaluations were performed blind to treatment.

No mortality occurred during the course of the study. No treatment-related clinical signs were observed. Body weight gain and food consumption were not adversely affected. No treatment-related findings were detected at necropsy. Intrauterine growth and survival were unaffected by treatment. No treatment-related fetal malformations or developmental variations were observed. The no-effect level (NOEL) for maternal toxicity and developmental toxicity was 1,000 mg/kg/day, the highest dose tested (*Stump, D. 1999. A Prenatal Developmental Toxicity Study of Hexabromocyclododecane (HBCD) in Rats. Laboratory Study No.: WIL-186009. WIL Research Laboratories, Inc., Ashland, OH.*).

4.4.4.2 Rat Developmental Toxicity Study

Murai et al. 1985 (*Pharmacometrics (Japan)* 29(6):981-986) identified no reproductive or developmental effects in the rat at doses up to 1% in the diet administered from days 0-20 of gestation. This dose is approximately equivalent to 500 mg/kg/d.

The Murai et al study consisted of a 7 day dose range finding study (n=5 rats/dose group) and a combined teratogenicity-developmental study (n=20/dose group). Doses in the 7 day range finding study were 0, 0.3, 1, 3 or 10 g/kg/day. Doses as high as 10 g/kg/day produced no evidence of toxicity. A statistically significant (P<0.01) increase in liver weight was noted in groups receiving ≥ 1 g/kg/day. Doses for the combined teratogenicity-developmental study were based on this increase in liver weight. In the combined teratogenicity-developmental study, pregnant female rats were fed diets containing 0, 0.01, 0.1, or 1% HBCD on days 0-20 of gestation. Daily doses were estimated by the authors to be 0, 5, 50 or 500 mg/kg/day and the average total dose/rat/group was estimated to be 0, 0.13, 1.28 or 12.0 g/kg. Rats were observed daily and body weight and food consumption measured. Fourteen rats from each group were sacrificed on day 20 of gestation and their fetuses were examined for toxicity or teratogenicity. Approximately 150 fetuses/dose level were examined for evidence of teratogenicity. All fetuses from all litters were examined for signs of external anomalies. Approximately 2/3 of the fetuses/dam were examined for skeletal abnormalities; the remaining fetuses from each dam were examined for any abnormalities of the internal organs. In addition, six rats from each group were allowed to deliver their litters and growth of the litters was observed until the 7th week post-parturition.

The authors' estimated the doses in the feed were equivalent to 0, 5, 50 or 500 mg HBCD /kg body weight /day. No adverse effects were detected in any treatment group with respect to maternal weight gain, food consumption, or gross appearance of internal organs. The mean liver (absolute and relative to body weight) weight in the 1% group was statistically different (higher) from the control mean. Normal development was seen in neonates carried through to six weeks of age.

There was no adverse effect of treatment on the number of corpora lutea, implants, resorptions, live fetuses, sex ratio, or body or placental weight. No fetal deaths occurred in any group. No external, skeletal or visceral malformations were detected. A few skeletal variations were detected but where of similar types and numbers in the control and treated groups.

There was no significant differences between the control and treated groups in the number of implantation, live newborns, dead newborns, live newborn parturition index. The weaning and survival index was comparable in the control and treated groups. Body weight changes in the newborns was comparable in all groups.

No reproductive or developmental effects were detected in rats at HBCD doses up to 1% in the diet (~500 mg/kg/d) administered from days 0-20 of gestation. Further, normal development was seen in neonates carried through to six weeks of age.

Dose levels: 0, 0.01, 0.1, or 1% HBCD on days 0-20 of gestation [Murai estimate: 0, 5, 50 or 500 mg/kg/day]. No teratogenic effects. Normal development in neonates carried through age 6 wks. NOEL = 1% of diet (Murai, T. Kawasaki, H., Kanoh, S. 1985.

Studies on the toxicity of insecticides and food additives in pregnant rats - fetal toxicity of Hexabromocyclododecane. Pharmacometrics (Japan) 29(6):981-986).

4.4.5 Reproductive Toxicity Data

Two teratology studies on HBCD are available; one published in the literature (high dose = 1% of the diet) and one recently completed by industry under current guidelines and Good Laboratory Practices using the HBCD in commercial production and use (high dose = 1,000 mg/kg/d). Both studies are negative for developmental toxicity. Repeated dose studies (two 28-day studies, one 90-day study, and one 18-month study in a second species) indicate HBCD does not affect the reproductive organs at doses up to 1,000 mg/kg/day. According to the SIDS Manual, when teratology and 90-day studies show no effects on the reproductive system then the requirement for the reproductive endpoint are met. Teratology, 28-day, 90-day and 18-month studies all demonstrate HBCD has no effect on the reproductive system at the limit dose of 1,000 mg/kg/d.

In the 1997 90-day study, considered the most informative of the subchronic studies mentioned in the preceding paragraph, the following organs of the reproductive tract were weighed at the 90-day and recovery sacrifices: epididymides (total and cauda), ovaries (with oviducts), prostate, testes, and uterus and cervix. These organs plus the seminal vesicles, vagina and vas deferens were examined histologically in animals in the control and high dose groups. Vaginal smears for determination of the stage of estrus were obtained from all females once daily beginning study day 69 through the 90-day necropsy. An assessment of spermatogenesis was performed at the 90-day sacrifice by evaluating sperm motility/viability, morphology and epididymal and testicular sperm numbers and production rate.

No adverse effect on the estrous cycle was detected in females receiving doses as high as 1,000 mg/kg/d for 90 consecutive days. No adverse effect on spermeogenesis was detected in males receiving doses as high as 1,000 mg/kg for 90 days. No test-article related changes in weight or microscopic effects were noted in the organs of the reproductive tract with the sole exception of an increase in weight of the prostate at 1,000 mg/kg/d on day 90. Mean prostate weight was increased in the 1,000 mg/kg/d dose group compared to the control mean after 90 days of treatment (0.95, 0.99, 1.12, 1.25* g in the control, 100, 300, and 1,000 mg/kg/d, respectively). The mean prostate weight relative to mean body and brain weights was also statistically increased at the high dose on day 90. At the recovery sacrifice, the prostate weights in the control and high dose groups were not statistically different. No test article-related changes in the prostate were detected on microscopic exam at either sacrifice.

4.4.6 Additional Toxicology Data

4.4.6.1 Pharmacokinetics

There are least two pharmacokinetic studies were performed in Japan in the early 1980s, as well as one from Velsicol (1980). One Japanese study used gas chromatography for

the analyses and therefore the results are questionable (R. Arita et al. 1983). The other Japanese study reportedly used ¹⁴C-labelled material and may be of more value. The Velsicol study reported that HBCD was absorbed and metabolized extensively with ~86% eliminated in 72 hrs. The 2001 90 day study sponsored by BFRIP showed very different levels of the three stereoisomers from that administered in the test article. Based on this limited data, HBCD would appear to be well absorbed and metabolized prior to elimination, but it is unclear how and to what extent. The three stereoisomers are likely handled differently in the mammalian system, based on their relative concentrations in the test article vs. adipose tissue in the 2001 rat 90-day study.

HBCD's review in "Toxicological Risks of Selected Flame Retardants" (*National Academy Press, Washington, DC, 2000, pages 54-55*) summarizes the data on pharmacokinetics as follows:

"Toxicokinetics

No human data on the toxicokinetics of HBCD were located for any route. No toxicokinetic studies via the dermal or inhalation exposure routes were reported in experimental animals. However, in a report by Dean and Leong (1977), rats exposed dermally to a high dose of HBCD in saline experienced diarrhea and slight weight loss. This finding indicates that at least some absorption occurs via the dermal route.

In an unpublished study by Velsicol Chemical Corporation (1980), rats administered a single oral dose of 1.93 mg of radiolabeled HBCD eliminated 86% of the dose within 72 hr. (The total dose administered was 7-9 mg/kg body weight.) Absorption from the gastrointestinal tract reportedly occurred rapidly, with a half-life of 2 hr. However, the amount of the absorbed fraction was not reported. HBCD was reported to be rapidly metabolized and eliminated in the feces and urine following absorption, with 70% of the administered radioactivity eliminated in the feces and another 16% eliminated in the urine 72 hr after dosing. A two-compartment model was constructed, with non-adipose tissues in one compartment and adipose tissue in the other. Elimination from the adipose compartment was reported to be slower than elimination from the non-adipose compartment, although elimination half-times were not provided in the review. In another study by Arita et al. (Marcia Hardy, Albemarle Corporation, Pers. Commun., August 3, 1999), HBCD was orally administered to male Wistar rats (number not reported) in olive oil at 500 mg/kg-d for 5 d. HBCD was found to be present only in adipose tissue, and in none of the other organs examined (i.e., spleen, pancreas, liver, kidneys, and heart). HBCD was found to be excreted in the feces, with an average of 32-35% of the cumulative administered dose excreted. No HBCD was found in the urine. Although differences in study design, including the test vehicle and the analytic methods used, may account for some of the difference in the results, both studies by Velsicol Chemical Corporation (1980) and Arita et al. (Marcia Hardy, Albemarle Corporation, Pers. Commun., August 3, 1999) suggest that following acute oral doses, HBCD is rapidly absorbed from the gastrointestinal tract, distributed primarily to body fat, and eliminated rapidly, primarily in the feces."

Further details on these studies are as follows.

The excretion of HBCD in urine and feces as well as its distribution to various organs was investigated (R. Arita, K. Miyazaki and S. Mure. 1983. Metabolic test of hexabromocyclododecane. Department of Pharmacy, Hokkaido University Hospital. Japan). The test article was "Pyroguard SR-103", manufactured by Daiichi Kogyo Seiyaku K.K.

A fine suspension of SR-103 in olive oil was prepared by mixing well to homogeneity in a mortar. The dose was 500 mg/kg/d for 5 consecutive days (n=4 male Wistar rats). The dose volume was 100 mg/ml. Urine and feces were collected separately by housing the rats in glass cages. Twenty-four hours after the last administration, the rats were sacrificed and the spleen, liver, pancreas, kidneys, heart and fatty tissue collected. Gas chromatography with an FID detector was used for quantification. The limit of quantification was about 5 ug/ml in urine and about 20 ug/ml in the fecal and organ homogenates.

The average daily rate of excretion in the feces was 29-37% of the dose. The cumulative excretion was roughly constant at 32-35% with respect to the cumulative administered amount. Urinary excretion was not observed. No evidence for the presence of metabolites was observed in urine or feces.

The test article was detected only in the adipose tissue after dosing for 5 days. The level in adipose tissue was 0.3-0.7 mg/g fat.

A separate study with isolated intestinal loop (upper jejunum) indicated about 12% of the dose was detectable in the intestinal tissue and the amount remaining in the lumen loop "small".

In another study, a single oral dose (7-9 mg/kg) of ¹⁴C-HBCD was administered to male (n=2) and female (n=8) rats (C.C. Yu and Y. H. Atallah. Velsicol Chemical Corporation, Laboratory Report. September 17, 1980). Based on the starting material used in the synthesis and the melting point of the final product described in the report, the test article appeared to be composed of the gamma isomer. The rats were sacrificed 8, 24, 48 and 72 hours (females) and 48 hours (males) after dosing. One female rat served as control. Urine and feces were collected daily, and blood samples from 4 animals were collected during the first 24 hours. Tissue samples were collected at the time of sacrifice.

Total ¹⁴C-activity was determined via liquid scintillation, and the parent molecule and metabolites were distinguished by thin layer chromatography in extracts of feces and urine.

HBCD appeared to be well absorbed from the gastrointestinal tract and extensively metabolized prior to elimination in feces (primary route) and urine. The estimated absorption half-life was 2 hours; peak radioactivity was detected in blood 4 hours post-dosing. The pharmacokinetics of the gamma stereoisomer appeared to follow an open

two-compartment model. The central compartment was described as liver, lung, kidney, heart, muscle, gonads, uterus, spleen, and brain; the peripheral compartment was described as adipose tissue. Roughly 80->90% of the gamma stereoisomer was eliminated within 3 days following a single oral dose, with an apparent half-life of elimination of 27 hours. Only metabolites were detected in feces and urine - no parent molecule was detected. Thus, the gamma isomer appears to be extensively metabolized prior to excretion. In contrast, only unmetabolized gamma isomer was detected in adipose tissue.

In females at 8, 24, 48 and 72 hours post-dosing, the total ¹⁴C-activity detected in tissues of female rats was ~43, 24, 18 and 17% of the dose, respectively. In male rats at 48 hours post-dosing (the only time point investigated in males), the ¹⁴C-activity was ~10% of the dose. Similarly, that detected in feces from female rats was ~4, 65, 54 and 77% at 8, 24, 48 and 72 h. Urine contained 0.1, 6, 18 and 15% of the dose at the same time intervals. Feces from male rats at 48 hours contained ~94% of the dose, while urine from male rats contained ~15%. At 48 hours post-dosing approximately 81% of the dose was detected in feces and urine of female rats. Thus, at 48 hours post-dosing approximately 86% of the dose was recovered in the tissues, feces and urine from female rats whereas 119% was recovered from males. The lower 48-hour recovery from this group of female rats is largely accounted for by the lower fecal content (43%) of radioactivity collected during the 0-24 hours post-dosing. By 48 hours post-dosing, females sacrificed at 24 hours, had a total fecal ¹⁴C-content of 65% of the dose, and females sacrificed at 72 hours had a total fecal ¹⁴C-content of 62% of the dose. Substituting the average value, 63%, from these two groups for the 43% value used to calculate overall recovery, the total percent of the dose accounted for at 48 hours becomes feces (74%), urine (17%) and tissues (18%) or 109% of the dose. It appears likely that some unknown factor resulted in the lower 0-24 hour percent recovery from feces, and that percent of dose present in tissues, feces and urine 48 hours post-dosing is similar in male and female rats.

Caution is urged in interpreting this data due to the small sample size and the brief nature of the final report.

4.4.6.2 Carcinogenicity: 18-Month Mouse Carcinogenicity

Male and female mice were fed diets containing HBCD at 0, 100, 1000 or 10,000 ppm for 18 months. There was no evidence of carcinogenicity at any dose level. This study was performed by the Department of Toxicology, National Public Health Research Institute, Biological Safety Test and Research Center, Japan (date not specified). Only a summary of the study is available.

The test substance was the commercial HBCD product from Daiichi Kogyo Seiyake K.K. There were 50 males and 50 females per dose level.

There was no effect of test article on mortality, clinical signs, body weights or food consumption. A variety of gross lesions/nodules were detected at necropsy, which were

not correlated with administration of test article. The main histopathologic changes noted were hepatocyte swelling, degeneration, necrosis, vacuole formation and fatty infiltration. These changes were suspected to be related to hepatic enzyme induction. There was no correlation of these changes with dose level. Various tumors were observed in many organs, but the incidence was sporadic and not associated with test article administration.

4.4.6.3 Skin Sensitization

Five sensitization studies have been conducted; three in guinea pigs, one in mice, and one in human volunteers. The 1997 guinea pig maximization test performed by BFRIP was negative. The Momma et al. (*Pharmacometrics*, 1985, 29:981-986) and Nakamura et al. (*Contact Dermatitis*, 1994, 31:72-85) studies reported in the literature were positive; the test article appears to have been an HBCD product produced in Japan. The patch test in human volunteers was negative. The 2003 mouse local lymph node assay, performed by BFRIP to clarify these results, was negative. Based on this data, the HBCD produced by its three major manufacturers is not a skin sensitizer.

4.4.6.3.1 1972 Human Patch Test (DuPont)

The test samples were Tyvek T-12 with 10% HBCD. One inch squares of the test samples were applied to the arms of 10 men and to the arms or legs of ten women and held in place with Dermicel tape for six days. After a two-week rest period, new patches were applied for 48 hours as a challenge test for skin sensitization. Skin under the patches was examined at two and six days after the first application and on removal of the challenge patch. No skin reactions were observed on any subject at any examination (McDonnell, M. 1972. *Haskell Laboratory Report No. 185-72. Haskell Laboratory for Toxicology and Industrial Medicine*).

4.4.6.3.2 Guinea Pig Skin Sensitization Tests

The 1997 Guinea Pig Maximization Skin Sensitization Test performed by BFRIP used a test article which was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. The study was conducted according to EPA, OECD and GLP guidelines. The test article used in this study was representative of the HBCD commercial product sold in the U.S. The test was negative for the induction of skin sensitization (Wenk M. 1996. *Maximization Test in Guinea Pigs. Test Article: Hexabromocyclododecane. Project No. M96AO61.1X64. Microbiological Associates, Inc. Rockville, MD*).

The Momma (1985) and Nakamura (1994) studies, which produced positive results, used an HBCD product manufactured in Japan.

The reason for the discrepancy between these results is not apparent. However, the negative results in the 1997 test that used the highest possible concentration for topical induction and challenge, raise questions about the potential for HBCD to produce even a

mild sensitization reaction in humans. The methodologies used in these 3 sensitization tests are provided in Table 4.

Table 4. Comparison of the methodology used in 3 guinea pig skin sensitization studies conducted on HBCD.

	BFRIP 1997	Momma et al. 1985	Nakamura et al. 1994
INDUCTION – ID			
VOLUME	0.1 ml	0.05 ml	Assume 0.05 ml ?
CONCENTRATION	5%	0.05, 0.5, 5%	0.5, 5%
DOSE	0.005 mg	0.000025, 0.00025, 0.0025 mg	0.00025, 0.0025 mg
VEHICLE	Corn oil	Olive oil	Olive oil
INDUCTION –TOPICAL			
AMOUNT	500 mg	200 mg	Assume 200 mg ?
CONCENTRATION	100%	25%	25%
DOSE	250 mg	50 mg	50 mg
VEHICLE	Corn oil*	Vaseline	Petrolatum
CHALLENGE			
VOLUME/AMOUNT	500 mg	0.02 ml	0.1 ml
CONCENTRATION	100%	0.005, 0.05, 5%	0.05, 0.5, 5%
DOSE	250 mg	0.000001, 0.00001, 0.0001, 0.001 mg	0.00005, 0.0005, 0.005 mg
VEHICLE	Corn oil*	Acetone	Acetone

*Only moistened with corn oil.

4.4.6.3.3 Mouse Local Lymph Node Assay (BFRIP and Dow Chemical Company)

The test article was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc. The study was conducted according to EPA, OECD and GLP guidelines.

The Local Lymph Node Assay (LLNA) assesses the potential of test materials to cause contact sensitization by measuring the lymphocyte proliferative responses from auricular lymph nodes following topical application of the test materials to mouse ears. Test materials that elicit a Stimulation Index (SI) of ≥ 3 (*i.e.*, 3- fold greater proliferation than control animals) should be considered positive for dermal sensitization potential. This test was performed to clarify the divergent results in the guinea pig maximization tests.

All mice received one of three concentrations of HBCD (2%, 20% or 50% w/v) or DMF (dimethylformamide) on days 1-3 (n=6 mice/group). HCA (α -hexyl cinnamaldehyde), a moderate contact sensitizer, was evaluated concurrently as a positive dermal sensitization control. The test materials were administered to the dorsal surface of both ears (25 μ l/ear). On day 6, all mice received an intravenous tail vein injection of phosphate

buffered saline containing 20 µCi of ³H-thymidine. Uptake into the auricular lymph nodes draining the site of chemical application was measured 5 hours later.

Body weight data were unremarkable and minor increases in ear thickness were noted suggesting slight irritation following applications of 20% and 50% HBCD. There were no indications that HBCD possesses dermal sensitization potential. SI values were consistently around 1.0 at all doses tested. Lymphocyte proliferation by DMF, vehicle treated mice (2015 dpm) was higher than historical laboratory values commonly observed using acetone and olive oil as a vehicle. This is not inconsistent with that reported in the literature for this vehicle. HCA administrations (30% v/v) elicited proliferation that was 3-fold greater than that of vehicle controls thus detecting the moderate contact sensitization potential in this study. On the basis of these results, HBCD would not be considered to have contact sensitization potential. (*Woolhiser M. 3003. Hexabromocyclododecane: Contact Sensitization Potential Via The Local Lymph Node Assay (Including A Primary Irritancy Screen) Using CBA/J Mice. Study Number 031013. Draft Final Report. Toxicology & Environmental Research And Consulting. The Dow Chemical Company. Midland, MI.*)

5.0 HBCD TESTING PLAN

A complete set of SIDS-level data currently exists for HBCD (Table 5), and the results are described in the attached robust summaries. Therefore, no testing is planned under this program.

Table 5. HBCD Test Plan Summary.

Study Type	Data Available	Data Acceptable	Estimation	Testing Required
Physical/Chemical				
Melting Point	Y	Y	-	N
Boiling Point	N	-	-	N
Vapor Pressure	Y	Y	-	N
Water Solubility	Y	Y	-	N
Environmental Fate				
Photodegradation	N	-	Y	N
Stability in Water	N	-	Y	N
Biodegradation	Y	Y	-	N
Transport (Fugacity)	N	-	Y	N
Ecotoxicity				
Acute Toxicity to Fish	Y	Y	-	N
Acute Toxicity to Aquatic Invertebrates	Y	Y	-	N
Toxicity to Aquatic Plants	Y	Y	-	N
Toxicology Data				
Acute Toxicity	Y	Y	-	N
Repeated Dose Toxicity	Y	Y	-	N
Genetic Toxicity – Mutation	Y	Y	-	N

Genetic Toxicity – Chromosome Aberration	Y	Y	-	N
Developmental Toxicity	Y	Y	-	N
Reproductive Toxicity	Y	Y	-	N
