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**HPV**  
**DATA SUMMARY AND TEST PLAN**  
**FOR**  
**HEXABROMOCYCLODODECANE (HBCD)**  
**CAS No. 3194556**

**Prepared by**

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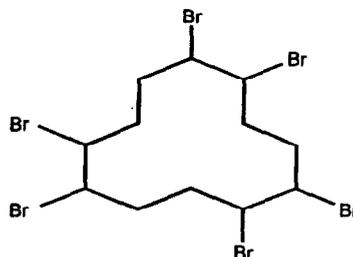
**December 20, 2001**

## 1.0 INTRODUCTION

The Brominated Flame Retardant Industry Panel (BFRIP) was formed in the 1980s to address issues related to the brominated flame retardants that its members manufacture in common, conduct research, and interact with regulatory agencies and other interested parties. Its members, who are global manufacturers of brominated flame retardants, are Albemarle Corporation, Ameribrom Inc. (a subsidiary of Dead Sea Bromine Group), and Great Lakes Chemical Corporation. Akzo-Nobel is an associate member. BFRIP, organized under the American Chemistry Council, volunteered under the U.S. EPA's High Production Volume (HPV) program to prepare the Data Summary/Test Plan and Robust Summaries for hexabromocyclododecane (HBCD). As discussed below, HBCD is a data-rich chemical, including valid studies or other information on all SIDS endpoints. For this reason, no additional tests are proposed for the purpose of this program.

## 2.0 HBCD's STRUCTURE AND PROPERTIES

HBCD, a solid at room temperature, is a cyclic aliphatic flame retardant (Fig. 1) with a molecular weight of 641.7. The commercial product is a mixture of three stereoisomers, alpha, beta and gamma, which are typically present at approximately 6, 8, and 80%, respectively.



**Figure 1. Hexabromocyclododecane (HBCD).**

The measured physical/chemical properties of the commercial HBCD product are as follows: water solubility 3.4 ug/L at 25°C (Stenzel, J. and Markley, B. 1997), vapor pressure  $6.27 \times 10^{-5}$  Pa at 21°C (Stenzel, J. and Nixon, W. 1997), and log octanol-water partition coefficient 5.625 at 25°C (MacGregor, J. and Nixon, W. 1997). The test article used for these measurements was a composite of 3 lots of commercial HBCD produced by Albemarle Corporation, Great Lakes Chemical Corporation, and Ameribrom, Inc., and the studies were conducted according to EPA, OECD and GLP guidelines. The product's melting point is ~ 186°C (range: 175-195°C) (Albemarle, 2001).

## 3.0 HBCD APPLICATIONS

HBCD is used as a flame retardant. Its primary application is in extruded (XPS) and expanded (EPS) polystyrene foam that is used as thermal insulation in the building

industry. HBCD is highly efficient in this application so that very low levels are required to reach the desired flame retardancy. Typical HBCD levels in EPS are 0.67% and in XPS 2.5%. At present, HBCD is the only suitable flame retardant 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 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 acceptable. HBCD is not used to flame retard electronic housings (e.g. television sets) that must meet the higher V-0 flame retardancy standard.

#### 4.0 HBCD TOXICOLOGY DATA SUMMARY

##### 4.1 ENVIRONMENTAL FATE (BFRIP)

HBCD's measured and predicted environmental fate parameters are provided in Table 1.

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*). 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 =  $1 \times 10^7$ ) (*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.

##### 4.1.1 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 ( $6.27 \times 10^{-5}$  Pa at 21°C) that would preclude substantial levels in the air.

##### 4.1.2 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).

#### 4.1.3 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*).

#### 4.1.2 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 ECOTOXICOLOGY DATA

HBCD was not acutely toxic to fish, daphnia or freshwater or marine alga at the limits of its water solubility. HBCD was not chronically toxic to daphnia nor was it toxic to fish early life stages at the limits of its water solubility. HBCD was bioconcentrated in fish.

### 4.2.1 Acute Toxicity to Fish

#### 4.2.1.1 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. HBCD's 96 hour LC50, no mortality concentration and no observed effect concentration were all > than HBCD's water solubility. The highest nominal dose tested was twice HBCD's water solubility.

**TABLE 1. 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 = $4.93 \times 10^{-3}$ 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 = $5.0 \times 10^{-12}$ cm <sup>3</sup> /molecule-sec Half-Life = 2.1 Days (12-hr day; $1.56 \times 10^{+6}$ OH/cm <sup>3</sup> ) Half-Life = 25.6 Hrs
Henry's Law Constant	Estimated (EPI win, V.3.04)	$6.4 \times 10^{-11}$ atm-m <sup>3</sup> /mole at 25 °C $2.6 \times 10^{-9}$ 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)	$1.1 \times 10^{+7}$
Volatization 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	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%
Level III 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 $9.9 \times 10^{-15}$ , Water $2.7 \times 10^{-18}$ , Soil $4.1 \times 10^{-20}$ , Sediment $2.6 \times 10^{-18}$  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 (CMA BFRIP 1996)	Not readily biodegradable

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).

#### 4.2.1.2 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).

#### 4.2.2 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 HBCD's water solubility (3.4ug/L measured). The highest nominal dose tested was twice 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).

#### 4.2.3 Toxicity to Aquatic Plants

##### 4.2.3.1 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. The highest nominal dose tested was twice 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).

#### 4.2.4.3 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).

#### 4.2.5 Prolonged Exposure Data

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

##### 4.2.5.1 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 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 residues of the alpha and beta diastereomers were evident 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 HBCD at mean measured concentrations up to 3.7 ug/L (nominal concentration = 6.8 ug/L or twice 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 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 HBCD's water solubility) (Drottar et al. 2001. *Hexabromocyclododecane (HBCD): An early life-stage toxicity test with the rainbow trout (Oncorhynchus mykiss)*. Project No.: 439A-112. Wildlife International, Ltd. Easton, MD).

#### 4.2.5.2 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. These doses are equivalent to 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. 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).

#### 4.2.5.3 *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 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. 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 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 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 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.

#### 4.3.1 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*).

#### 4.3.2 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.

##### 4.3.2.1 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 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).*

#### 4.3.2.2 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 (T<sub>3</sub>, T<sub>4</sub> 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 generally secondary to the inducing effects on the liver or were otherwise not considered adverse effects of treatment as discussed further below.

Statistically significant ( $p < 0.05$ ) test article-related 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; furthermore, these serum albumin and gamma glutamyltransferase increases were probably secondary to the increases in liver weight. The increases in serum chloride were probably secondary to presence of free bromide in the test article preparation which interfered with the chloride determination methodology. The decrease in  $T_4$ , which was also reversible, was also probably secondary to increased liver weight (secondary to microsomal enzyme induction, known to cause increased metabolism and clearance of  $T_4$  in the rat).

The incidence of observations noted at gross necropsy was low and there was no evidence of frank organ damage. On histopathologic examination of tissues, relatively mild findings occurred in both the control and treated groups. Potential test article-related histologic changes were identified in the liver and thyroid glands but these would not be considered indicative of frank toxicity. These organs were examined microscopically in all groups at both necropsies. 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. Minimal hepatocellular vacuolation was also detected in females in the control and test article treated groups without a clear dose response (3 to 4/10 animals per group) but, 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 increases in liver weight were a result of a microsomal enzyme inducing effect and were not typically considered indicative of toxicity in absence of frank organ damage. The reversible histologic changes (vacuolation and hypertrophy) are often found to accompany increased liver weight caused by liver enzyme induction. 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. The histologic changes in the thyroid had also nearly completely resolved except in the 1000 mg/kg/day group females, where partial recovery occurred.

Increases in mean prostate weight were noted in the 1000 mg/kg/day group males at the primary necropsy. However, the increases in prostate weight were probably not of toxicological significance since the increases did not persist to the recovery period, there were no correlating histologic findings and no change in sperm production.

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 additional findings observed at 1000 mg/kg/day (increased gamma glutamyltransferase and additional increases in the size of the liver and prostate), were also reversible, not associated with specific target organ damage or diminished function and were, therefore, probably of limited, if any, toxicologic significance. On this basis the no-observed-adverse-effect level (NOAEL) of HBCD administered to CrI:CD<sup>®</sup>(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*).

#### 4.3.2.3 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). This paper 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."

#### 4.3.2.4 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*).

### 4.3.3 Genetic Toxicity – Mutation

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

#### 4.3.3.1 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.*)

#### 4.3.3.2 *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*).

#### 4.3.3.3 *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  $1 \times 10^5$  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.

#### 4.3.4 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*).

#### 4.3.5 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.

##### 4.3.5.1 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.*).

#### 4.3.5.2 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  $\geq 1$  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 were 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*).

#### 4.3.6 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 = 1000 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 1000 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 1000 mg/kg/d.

## 4.3.7 Additional Toxicology Data

### 4.3.7.1 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 <sup>14</sup>C-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.

### 4.3.7.2 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).

### 4.3.7.3 Skin Sensitization

Four sensitization studies have been conducted; three in guinea pigs 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.

#### 4.3.7.3.1 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*).

#### 4.3.7.3.2 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 2.

**TABLE 2. Comparison of the methodology used in 3 guinea pig skin sensitization studies conducted on HBCD.**

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

\* Only moistened with corn oil.

## 5.0 HBCD TESTING PLAN

A complete set of SIDS-level data currently exists for HBCD (Table 3), and the results are described in the attached robust summaries. Therefore, no testing is planned under this program.

**TABLE 3. HBCD Test Plan Summary.**

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