EPA/630/R-13/235
Preliminary Materials
www.epa.gov/iris

Preliminary Materials for the Integrated Risk Information System (IRIS) Toxicological Review of Hexabromocyclododecane (HBCD)

[CASRN 3194-55-6]

DRAFT

March 2014

NOTICE

This document is comprised of **preliminary materials**. This information is distributed solely for the purpose of pre-dissemination review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. It is being circulated for review of its technical accuracy and science policy implications.

National Center for Environmental Assessment
Office of Research and Development
U.S. Environmental Protection Agency
Washington, DC

DISCLAIMER

This document is comprised of preliminary materials for review purposes only. This information is distributed solely for the purpose of pre-dissemination review under applicable information quality guidelines. It has not been formally disseminated by EPA. It does not represent and should not be construed to represent any Agency determination or policy. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

CONTENTS

TABLES

FIGURES

PREFACE

This draft document presents a planning and scoping summary, information on the approaches used to identify pertinent literature and primary studies, results of the literature search, approaches for selection of studies for hazard identification, presentation of characteristics and information from primary studies in evidence tables and exposure-response arrays, and mechanistic information in a summary table for hexabromocyclododecane (henceforth referred to as HBCD) prepared under the auspices of EPA's Integrated Risk Information System (IRIS) Program. This material is being released for public viewing and comment prior to a public meeting, providing an opportunity for the IRIS Program to engage in early discussions with stakeholders and the public on data that may be used to identify adverse health effects and characterize dose-response relationships.

The planning and scoping summary includes information on the uses of HBCD, occurrence of HBCD in the environment, and the rationale and scope for the development of the assessment. This information is responsive to recommendations in the 2009 National Research Council (NRC) report *Science and Decisions: Advancing Risk Assessment* (NRC, 2009) related to planning and scoping in the risk assessment process.

The preliminary materials are also responsive to the NRC 2011 report *Review of the Environmental Protection Agency's Draft IRIS Assessment of Formaldehyde* (NRC, 2011). The IRIS Program's implementation of the NRC recommendations is following a phased approach that is consistent with the NRC's "Roadmap for Revision" as described in Chapter 7 of the formaldehyde review report. The NRC stated that "the committee recognizes that the changes suggested would involve a multi-year process and extensive effort by the staff at the National Center for Environmental Assessment and input and review by the EPA Science Advisory Board and others." Phase 1 of implementation has focused on a subset of the short-term recommendations, such as editing and streamlining documents, increasing transparency and clarity, and using more tables, figures, and appendices to present information and data in assessments. Phase 1 also focused on assessments near the end of the development process and close to final posting. Phase 2 of implementation is focused on assessments that are in the beginning stages of assessment development. The IRIS HBCD assessment is in Phase 2 and represents a significant advancement in implementing the NRC recommendations. In the development of this assessment many of the recommendations are being implemented in full, while others are being implemented in part. Achieving full and robust implementation of certain recommendations will be an evolving process with input and feedback from the public, stakeholders, and independent external peer review. Phase 3 of implementation will incorporate the longer-term recommendations made by the NRC, including the development of a standardized approach to describe the strength of evidence for

noncancer effects. On May 16, 2012, EPA announced[1] that as a part of a review of the IRIS Program's assessment development process, the NRC will also review current methods for weight-of-evidence analyses and recommend approaches for weighing scientific evidence for chemical hazard identification. This effort is included in Phase 3 of EPA's implementation plan.

The literature search strategy, which describes the processes for identifying scientific literature, screening studies for consideration, and identifying primary sources of health effects data, is responsive to NRC recommendations regarding the development of a systematic and transparent approach for identifying the primary literature for analysis. The preliminary materials also describe EPA's approach for the selection of primary studies to be included in the evidence tables, as well as the approach for evaluating methodological features of studies that will be considered in the overall evaluation and synthesis of evidence for each health effect. The development of these materials is in response to the NRC recommendation to thoroughly evaluate critical studies with standardized approaches that are formulated and based on the type of research (e.g., observational epidemiology or animal bioassays). In addition, NRC recommendations for standardized presentation of key study data are addressed by the development of the preliminary evidence tables and preliminary exposure-response arrays for primary health effect information, and summary tables for mechanistic data.

EPA welcomes all comments on the preliminary materials in this document, including the following:

- the clarity and transparency of the materials;
- the approach for identifying pertinent studies;
- the selection of primary studies for data extraction to preliminary evidence tables and exposure-response arrays;
- any methodological considerations that could affect the interpretation of or confidence in study results; and
- any additional studies published or nearing publication that may provide data for the evaluation of human health hazard or dose-response relationships.

The preliminary evidence tables and exposure-response arrays should be regarded solely as representing the data on each endpoint that have been identified as a result of the draft literature search strategy. They do not reflect any conclusions as to hazard identification or dose-response assessment.

After obtaining public input and conducting additional study evaluation and data integration, EPA will revise these materials to support the hazard identification and dose-response assessment in a draft Toxicological Review that will be made available for public comment.

[1] EPA Announces NAS' Review of IRIS Assessment Development Process. 05/16/2012. http://yosemite.epa.gov/opa/admpress.nsf/0/1ce2a7875daf093485257a000054df54?OpenDocument

1. PLANNING AND SCOPING SUMMARY

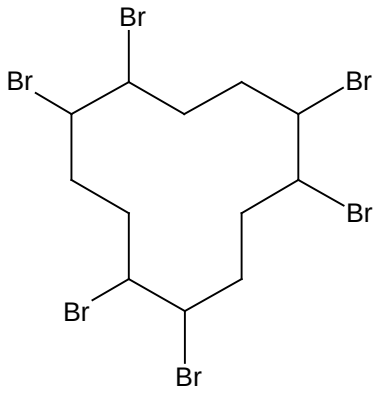
1.1. HBCD Chemistry and Uses

Flame retardant chemicals are used in a variety of products to reduce fire risks. HBCD has been used to treat multiple products, including textiles, polystyrene thermal insulation and certain polystyrene plastic used for electronic devices, and others. In November of 2014, the listing of HBCD as a Persistent Organic Pollutant (POP) under the Stockholm Convention will take effect, and the allowable uses of HBCD will be restricted. Uses in polystyrene insulation will be allowed to continue under the Convention until 2019 when alternatives are expected to be available for this use.[2] In 2012, EPA proposed a rule under the Toxic Substances Control Act to restrict uses of HBCD.[3]

Previous uses of HBCD, such as on textiles, may have led to greater human exposure than insulation uses.[4] In polystyrene insulation, HBCD is added to the polymer at high temperature and/or pressure. The material is not chemically bound to the polystyrene, however its diffusion from insulation foam is expected to be low.[5] Although HBCD releases to the human environment may be attenuated through changes in use, hundreds of millions of pounds of the material have been used and are present in treated products at varying lifecycle stages. Given continued HBCD use and the persistence of the chemical, the potential for human exposure is likely to continue for many years.

HBCD is not a naturally occurring chemical. The technical product is generally reported to exceed 94% purity with detected impurities including tetrabromocyclododecane and part per billion levels of polybrominated dibenzofurans.[6] The chemical HBCD can exist in 16 isomeric forms. It may be designated as a mixture of all isomers (CASRN 25637-99-4) or as a mixture of three main diastereomers when the bromine atoms are in the 1, 2, 5, 6, 9 and 10 positions (CASRN 3194-55-6). Commercial HBCD contains primarily a mixture of the three major diastereomers, termed α, β and γ. Four commercial grades of HBCD have been used and vary in proportions of the α, β and γ diastereomers: low melt, medium range, high melt and thermally stabilized.[7]

[2] http://cen.acs.org/articles/91/i19/Global-Ban-Flame-Retardant.html
[3] http://www.reginfo.gov/public/do/eAgendaViewRule?pubId=201310&RIN=2070-AJ88
[4] http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=7882C148-1#a3
[5] http://www.epa.gov/dfe/pubs/projects/hbcd/hbcd-draft-full-report.pdf
[6] European Commission Risk Assessment for Hexabromocyclododecane (http://esis.jrc.ec.europa.eu/doc/risk_assessment/REPORT/hbcddreport044.pdf)
[7] Environment Canada Screening Assessment Report for Hexabromocyclododecane (http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=7882C148-1#a1)



1,2,5,6,9,10-Hexabromocyclododecane
($C_{12}H_{18}Br_6$; CASRN 3194-55-6)

1.2. HBCD in the Environment

In water and soil, HBCD is generally understood to degrade slowly. Environmental half life estimates from field studies suggest half lives exceeding one year. HBCD has low water solubility and binds to sediment or suspended solids in aquatic environments. In soil, it binds strongly to soil organic matter, which reduces the amount that can leach into groundwater. Because it is semivolatile, HBCD exposed to air can partition into the atmosphere. The volatility and environmental persistence of HBCD account for its detection far from use and waste sites (i.e., the arctic).

In ecosystems, HBCD is reported to bioconcentrate and biomagnify. Consistent with HBCD's reported potential to bioaccumulate, the chemical is on several state and international priority lists[8]:

- the Canadian Environmental Protection Act Environmental Registry Domestic Substances List as Persistent, Bioaccumulative, and Inherently Toxic;
- Washington Department of Ecology's Persistent, Bioaccumulative, Toxic Chemicals in Washington State's Administrative Code;
- the European Commission as persistent, bioaccumulative and toxic in the candidate list of Substances of Very High Concern;
- the California Environmental Contaminant Biomonitoring Program as a Priority Chemical
- the Stockholm Convention as a Persistent Organic Pollutant

HBCD has been detected in human breast milk, adipose tissue and blood. HBCD was measured in fetal liver tissue at concentrations ranging from below the detection limit to 4,500 ng/g of lipid.[9] In homes, HBCD has been detected in air and dust. Concentrations reported in

[8] http://www.dtsc.ca.gov/SCP/upload/Group-Member-Candidate-Chemicals-List.pdf
[9] http://www.sciencedirect.com/science/article/pii/S0048969713009285#

fish have been as high as 8,000 ng/g of lipid but most reported levels are below 50 ng/g of lipid. An estimate of human dietary intake in the U.S. is reported to be 15.4 ng/day.[10]

A 2008 European risk assessment found that exposure to indoor air and airborne dust in homes was an insignificant route of exposure and focused more on dietary exposure.[11] However, other researchers have suggested that while diet is an important route of exposure, inhalation and ingestion of dust are increasingly being considered to be the major sources of human exposure.[12]

Release to the environment during the manufacturing process is considered to be low.[13] To reduce releases of HBCD during the manufacturing process, some facilities have put in place dust filtering systems, catalytic burning systems, wastewater treatment systems involving activated carbon and/or biomembrane reactors, and specialized waste incineration processes.[14]

1.3. Rationale for the Development of the Toxicological Review

Given its potential for widespread human exposure, the IRIS Program is developing an assessment of HBCD to address multiple needs. Several activities that would benefit from the IRIS assessment of HBCD are presented below:

- Due to concerns associated with HBCD exposure and toxicity, EPA has identified HBCD as a priority and released a detailed Action Plan announcing several rulemakings being considered under the Toxic Substances Control Act and the Toxics Release Inventory.[15] An HBCD IRIS assessment would provide useful information for rulemaking and HBCD health risk assessment.
- EPA is also reviewing alternatives for major HBCD uses through the Design for the Environment Program (DfE).[16] DfE evaluates chemicals' lifecycles and assesses risks of potential replacement chemicals to identify feasible alternatives. An IRIS assessment of HBCD could help inform the DfE comparative toxicity and risk assessment for identifying safer alternatives. The information developed by DfE could encourage the use of safer chemicals and technologies.[17]
- EPA, the Food and Drug Administration (FDA) and states issue advice about consuming fish that may be unhealthy to eat due to contamination. IRIS assessments provide useful information on chemicals' toxicity for developing these advisories, and EPA guidance for developing fish advisories recommends that IRIS values be used in setting screening values

[10] http://www.ec.gc.ca/ese-ees/default.asp?lang=En&n=A167D02F-1#a11
[11] http://esis.jrc.ec.europa.eu/doc/risk_assessment/REPORT/hbcddreport044.pdf
[12] http://www.sciencedirect.com/science/article/pii/S0048969713009285#
[13] http://echa.europa.eu/documents/10162/13640/tech_rep_hbcdd_en.pdf
[14] http://www.bsef.com/uploads/Factsheet_HBCD_25-10-2012.pdf
[15] http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/hbcd.html#address
[16] http://www.epa.gov/dfe/pubs/projects/hbcd/about.htm
[17] http://www.epa.gov/oppt/existingchemicals/pubs/actionplans/hbcd.html#address

for consumption levels.[18] Because HBCD has been identified by several organizations as a toxic, persistent and bioaccumulating chemical, an IRIS assessment may inform whether advisories are warranted.

- HBCD was considered for inclusion on the third Contaminant Candidate List (CCL 3) under the Safe Drinking Water Act[19] but it was not included. EPA is required to update this list of water contaminants every five years and identify those contaminants that may warrant future regulatory action. EPA uses a multi-step process to evaluate occurrence and health information to determine the substances that are included on the CCL. IRIS Reference Values, cancer dose-response information and cancer descriptors, when they are available, are used to evaluate health effects of potential CCL chemicals.
- IRIS values are also used in the development of Human Health Ambient Water Quality Criteria (HH-AWQC) under the Clean Water Act. A HH-AWQC is the highest concentration of a pollutant in water that is not expected to pose a significant risk to human health when considering ingestion of water and aquatic organisms or aquatic organisms only. These values are used by states in controlling discharges to ambient water bodies with "drinkable fishable" use designations.
- Given HBCD's level of use and its environmental persistence, an IRIS assessment is anticipated to be useful for EPA programs involved in waste management and site cleanup.
- HBCD has been identified as a Substance of Very High Concern (SVHC) under the European Registration, Evaluation, Authorisation and Restriction of Chemicals Program (REACH). As an SVHC, HBCD may become subject to the "authorisation" process to ensure less dangerous substances are used in HBCD replacement.

1.4. General Scope of the Toxicological Review

The Toxicological Review of HBCD will consider health effects data for cancer and noncancer endpoints from subchronic and chronic exposures to HBCD. Three broad types of studies, if available, will be used to inform human health effects: controlled human exposure, epidemiologic, and experimental studies. Mechanistic or mode of action data will be evaluated and may inform questions of human relevance, susceptibility, and dose-response relationships. Considering the potential uses of IRIS information and potential pathways of exposure, an IRIS assessment of HBCD would be expected to incorporate the following, provided that adequate data are available:

- Systematic identification of hazards from long-term exposures
- Analysis of mode of action information, if available

[18] http://water.epa.gov/scitech/swguidance/fishshellfish/techguidance/risk/upload/2009_04_23_fish_advice_volume1_v1cover.pdf

[19] http://water.epa.gov/scitech/drinkingwater/dws/ccl/upload/CCL3_Chemicals_Universe_08-31-09_508_v3.pdf

- Dose-response relationships for identified hazards
- Chronic Reference Concentration (RfC)
- Chronic Reference Dose (RfD)
- Cancer assessment and weight of evidence descriptor for oral and inhalation exposure, including dose-response information
- Identification of human populations and developmental stages with potentially greater susceptibility to HBCD

The HBCD assessment will rely on existing analytical tools and toxicity data and contain qualitative characterizations of uncertainty and variability related to hazard assessment and dose-response relationships. The development process for this assessment will provide opportunities for public comment and dialogue and includes independent external peer review.

2. DRAFT LITERATURE SEARCH AND SCREENING STRATEGY

The NRC (NRC, 2011) recommended that EPA develop a detailed search strategy utilizing a graphical display documenting how initial search findings are narrowed to the final studies that are selected for further evaluation on the basis of inclusion and exclusion criteria. Following these recommendations, a literature search and screening strategy was applied to identify literature related to characterizing the health effects of HBCD. This strategy consisted of a search of online scientific databases and other sources, casting a wide net in order to identify all potentially pertinent studies. In subsequent steps, references were screened to exclude papers not pertinent to an assessment of the health effects of HBCD, and remaining references were sorted into categories for further evaluation.

The literature search for HBCD was conducted in four online scientific databases, including PubMed, Toxline, Toxcenter, and TSCATS, in August 2013. The detailed search approach, including the search strings and number of citations identified per database, is presented in Table 2-1. This search of online databases identified 635 citations (after electronically eliminating duplicates). The computerized database searches were also supplemented by a review of online regulatory sources as well as "forward" and "backward" searches of Web of Science using several key references (Table 2-2); 29 citations were obtained using these additional search strategies. In total, 664 citations were identified using online scientific databases and additional search strategies.

These citations were screened using the title, abstract, and in limited instances, full text for pertinence to examining the health effects of HBCD exposure. The process for screening the literature is described below and is shown graphically in Figure 2-1.

- 41 references were identified as potential primary sources of health effects data and were considered for data extraction to evidence tables and exposure-response arrays.
- 118 references were identified as not being pertinent and were excluded from further consideration (see Figure 2-1 for exclusion categories).
- 39 references were kept for further review. This category includes references that did not provide enough material to evaluate pertinence (e.g., abstract not available).
- 357 references were identified as not primary sources of health effects data (e.g., reviews and studies with chemical/physical property information), but were kept as additional resources for development of the Toxicological Review.
- 109 studies were identified as supporting studies; these included 54 studies providing genotoxicity and other mechanistic information and 55 toxicokinetic studies. The 54

supporting studies with genotoxicity and other mechanistic information were considered for inclusion in a compendium of mechanistic study information.

The literature will be regularly monitored for the publication of new studies and a formal updated literature search and screen will be conducted after the IRIS bimonthly public meeting discussing these preliminary materials.

The documentation and results for the literature search and screen can be found on the Health and Environmental Research Online (HERO) website (http://hero.epa.gov/HBCD).[20]

[20] HERO (Health and Environmental Research On-line) is a database of scientific studies and other references used to develop EPA's risk assessments aimed at understanding the health and environmental effects of pollutants and chemicals. It is developed and managed in EPA's Office of Research and Development (ORD) by the National Center for Environmental Assessment (NCEA). The database includes more than 300,000 scientific articles from the peer-reviewed literature. New studies are added continuously to HERO.

It is important to note that the HERO database will be regularly updated as additional references are identified during assessment development. Therefore, the numbers of references (by tag) displayed on the HERO webpage for HBCD may not match the numbers of references identified in Figure 2-1 (current through March 2014).

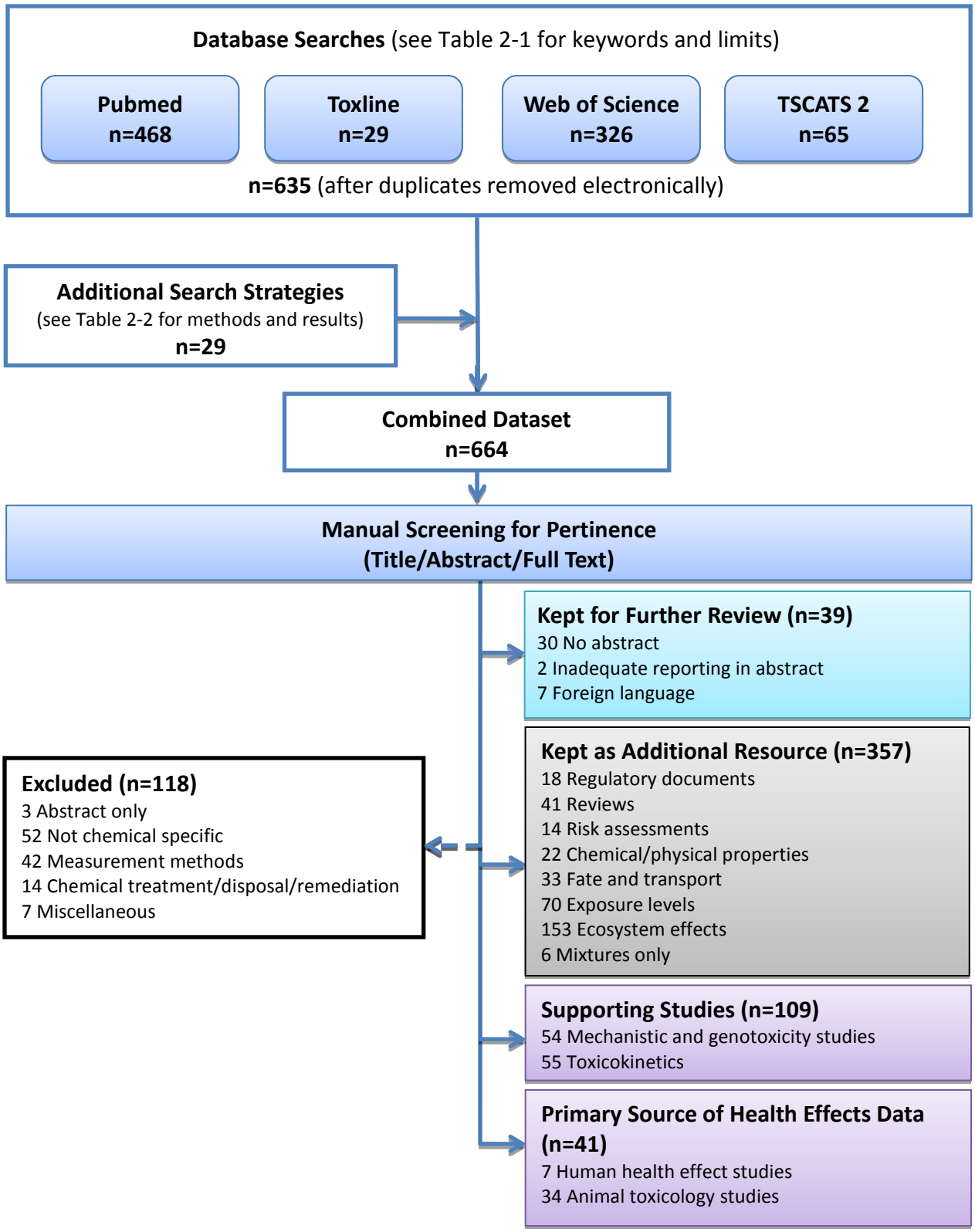


Figure 2-1. Summary of literature search and screening process for HBCD.

1 **Table 2-1. Summary of detailed search strategies for HBCD**

Database Search Date	Set #	Terms	Hits
PubMed 08/20/13	1A	hexabromocyclododecane[nm] OR "3194-55-6"[tw] OR "25637-99-4"[tw] OR "1,2,5,6,9,10-hexabromocyclodecane"[tw] OR hexabromocyclododecane*[tw] OR hbcd[tw] OR hbcds[tw]	468
Web of Science 08/21/13	1B1	(TS="1,2,5,6,9,10-hexabromocyclodecane" OR TS="hexabromocyclododecane" OR TS=hexabromocyclododecane* OR TS="HBCD" OR TS="HBCDs") AND ((WC=("Toxicology" OR "Endocrinology & Metabolism" OR "Gastroenterology & Hepatology" OR "Gastroenterology & Hepatology" OR "Hematology" OR "Neurosciences" OR "Obstetrics & Gynecology" OR "Pharmacology & Pharmacy" OR "Physiology" OR "Respiratory System" OR "Urology & Nephrology" OR "Anatomy & Morphology" OR "Andrology" OR "Pathology" OR "Otorhinolaryngology" OR "Ophthalmology" OR "Pediatrics" OR "Oncology" OR "Reproductive Biology" OR "Developmental Biology" OR "Biology" OR "Dermatology" OR "Allergy" OR "Public, Environmental & Occupational Health") OR SU=("Anatomy & Morphology" OR "Cardiovascular System & Cardiology" OR "Developmental Biology" OR "Endocrinology & Metabolism" OR "Gastroenterology & Hepatology" OR "Hematology" OR "Immunology" OR "Neurosciences & Neurology" OR "Obstetrics & Gynecology" OR "Oncology" OR "Ophthalmology" OR "Pathology" OR "Pediatrics" OR "Pharmacology & Pharmacy" OR "Physiology" OR "Public, Environmental & Occupational Health" OR "Respiratory System" OR "Toxicology" OR "Urology & Nephrology" OR "Reproductive Biology" OR "Dermatology" OR "Allergy")) OR (WC="veterinary sciences" AND (TS="rat" OR TS="rats" OR TS="mouse" OR TS="murine" OR TS="mice" OR TS="guinea" OR TS="muridae" OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS="dog" OR TS="dogs" OR TS=beagle* OR TS="canine" OR TS="cats" OR TS="feline" OR TS="pig" OR TS="pigs" OR TS="swine" OR TS="porcine" OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset*)) OR (TS=toxic* AND (TS="rat" OR TS="rats" OR TS="mouse" OR TS="murine" OR TS="mice" OR TS="guinea" OR TS="muridae" OR TS=rabbit* OR TS=lagomorph* OR TS=hamster* OR TS=ferret* OR TS=gerbil* OR TS=rodent* OR TS="dog" OR TS="dogs" OR TS=beagle* OR TS="canine" OR TS="cats" OR TS="feline" OR TS="pig" OR TS="pigs" OR TS="swine" OR TS="porcine" OR TS=monkey* OR TS=macaque* OR TS=baboon* OR TS=marmoset* OR TS="child" OR TS="children" OR TS=adolescen* OR TS=infant* OR TS="WORKER" OR TS="HUMAN" OR TS=patient*)) OR TS="exposure")	326
ToxLine 08/22/13	1C1	@OR+(@term+@rn+25637-99-4+@term+@rn+3194-55-6)+@NOT+@org+pubmed+pubdart+"nih+reporter"+tscats	22
	1C2	@OR+("hexabromocyclodecane"+"hexabromocyclododecane"+"hexabromocyclododecane"+"hexabromocyclododecanes"+"hbcd"+"hbcds")+@NOT+@org+pubmed+pubdart+"nih+reporter"+tscats	20
TSCATS 1 08/22/13	1D1	@term+@rn+25637-99-4+@AND+@org+tscats	12
	1D2	@term+@rn+3194-55-6+@and+@org+tscats	53
TSCATS 2	1E1	3194-55-6, 25637-99-4	10

08/22/13		date limited, 2000-date of search	
TSCA 8e/FYI recent submissions 08/22/13	1E1	Google: 3194-55-6, 25637-99-4 with (8e or fyi) tsca	4
Merged Reference Set	1	(duplicates eliminated through electronic screen)	635

1

2 **Table 2-2. Processes used to augment the search of core databases for HBCD**

System Used	Selected Key Reference(s) or Sources	Date	Additional References Identified
Manual search of citations from regulatory documents	European Commission. (2008). Risk Assessment: Hexabromocyclododecane. Final report. Luxembourg: Office for Official Publications of the European Communities	9/2013	7 citations added
	Environment Canada. (2011). Screening Assessment Report on Hexabromocyclododecane; Chemical Abstracts Service Registry Number 3194-55-6, Environment Canada, Health Canada	9/2013	0 citations added
Web of Science, forward search	Ema, M; Fujii, S; Hirata-Koizumi, M; Matsumoto, M. (2008). Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats. Reprod Toxicol 25: 335-351	9/2013	0 citations added
	Eriksson, P; Fischer, C; Wallin, M; Jakobsson, E; Fredriksson, A. (2006). Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD). Environ Toxicol Pharmacol 21: 317-322	9/2013	0 citations added
	Saegusa, Y; Fujimoto, H; Woo, GH; Inoue, K; Takahashi, M; Mitsumori, K; Hirose, M; Nishikawa, A; Shibutani, M. (2009). Developmental toxicity of brominated flame retardants, tetrabromobisphenol A and 1,2,5,6,9,10-hexabromocyclododecane, in rat offspring after maternal exposure from mid-gestation through lactation. Reprod Toxicol 28: 456-467	9/2013	0 citations added
	van Der Ven, LTM; van De Kuil, T; Leonards, PEG; Slob, W; Lilienthal, H; Litens, S; Herlin, M; Hãkansson, H; Cantón, RF; van Den Berg, M; Visser, TJ; van Loveren, H; Vos, JG; Piersma, AH. (2009). Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats. Toxicol Lett 185: 51-62	9/2013	0 citations added
Web of Science, backward search	Ema, M; Fujii, S; Hirata-Koizumi, M; Matsumoto, M. (2008). Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats. Reprod Toxicol 25: 335-351	9/2013	2 citations added

System Used	Selected Key Reference(s) or Sources	Date	Additional References Identified
	Eriksson, P; Fischer, C; Wallin, M; Jakobsson, E; Fredriksson, A. (2006). Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD). Environ Toxicol Pharmacol 21: 317-322	9/2013	1 citation added
	Saegusa, Y; Fujimoto, H; Woo, GH; Inoue, K; Takahashi, M; Mitsumori, K; Hirose, M; Nishikawa, A; Shibutani, M. (2009). Developmental toxicity of brominated flame retardants, tetrabromobisphenol A and 1,2,5,6,9,10-hexabromocyclododecane, in rat offspring after maternal exposure from mid-gestation through lactation. Reprod Toxicol 28: 456-467	9/2013	0 citations added
	van Der Ven, LTM; van De Kuil, T; Leonards, PEG; Slob, W; Lilienthal, H; Litens, S; Herlin, M; Hãkansson, H; Cantón, RF; van Den Berg, M; Visser, TJ; van Loveren, H; Vos, JG; Piersma, AH. (2009). Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats. Toxicol Lett 185: 51-62	9/2013	0 citations added
References obtained during the assessment process	Snowball search	9/2013	9 citations added
Background Check	Searched a combination of CASRNs and synonyms on the following databases: ACGIH (http://www.acgih.org/home.htm) ATSDR (http://www.atsdr.cdc.gov/substances/index.asp) CalEPA Office of Environmental Health Hazard Assessment (http://www.oehha.ca.gov/risk.html) Search this as well as the following sites (save the first 50 results) OEHHA Toxicity Criteria Database (http://www.oehha.ca.gov/tcdb/index.asp) Biomonitoring California-Priority Chemicals (http://www.oehha.ca.gov/multimedia/biomon/pdf/PriorityChemsCurrent.pdf) Biomonitoring California-Designated Chemicals (http://www.oehha.ca.gov/multimedia/biomon/pdf/DesignatedChemCurrent.pdf) Cal/Ecotox Database (http://www.oehha.ca.gov/scripts/cal_ecotox/CHEMLIST.ASP) OEHHA Fact Sheets (http://www.oehha.ca.gov/public_info/facts/index.html) Non-cancer health effects Table (RELs) http://www.oehha.ca.gov/air/allrels.html and Cancer Potency Factors (Appendix A and AppendixB)	8/26/2013	10 citations added

System Used	Selected Key Reference(s) or Sources	Date	Additional References Identified
	http://www.oehha.ca.gov/air/hot_spots/tsd052909.html CPSC (http://www.cpsc.gov) eChemPortal (participating databases: ACToR, AGRITOX, CCR, CCR DATA, CESAR, CHRIP, ECHA CHEM, EnviChem, ESIS, GHS-J, HPVIS, HSDB, HSNO CCID, INCHEM, J-CHECK, JECDB, NICNAS PEC, OECD HPV, OECD SIDS IUCLID, SIDS UNEP, UK CCRMP Outputs, US EPA IRIS, US EPA SRS) (http://www.echemportal.org/echemportal/participant/page.action?pageID=9) Environment Canada – Search entire site (http://www.ec.gc.ca/default.asp?lang=En&n=ECD35C36) if not found below: Toxic Substances Managed Under CEPA (http://www.ec.gc.ca/toxiques-toxics/Default.asp?lang=En&n=98E80CC6-1) Search results Final Assessments (http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&xml=09F567A7-B1EE-1FEE-73DB-8AE6C1EB7658) Draft Assessments (http://www.ec.gc.ca/lcpe-cepa/default.asp?lang=En&xml=6892C255-5597-C162-95FC-4B905320F8C9) EPA Acute Exposure Guideline Levels (http://www.epa.gov/oppt/aegl/pubs/chemlist.htm) EPA – IRISTrack/New Assessments and Reviews (http://cfpub.epa.gov/ncea/iristrac/) to find dates (http://www.epa.gov/ncea/iris/index.html) to find data EPA NSCEP (http://www.epa.gov/ncepihom/) EPA Science Inventory (http://cfpub.epa.gov/si/) FDA (http://www.fda.gov/) Federal Docket (www.regulations.gov) Health Canada First Priority List Assessments (http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl1-lsp1/index-eng.php) Health Canada Second Priority List Assessments (http://www.hc-sc.gc.ca/ewh-semt/pubs/contaminants/psl2-lsp2/index-eng.php) IARC Index: http://monographs.iarc.fr/ENG/Monographs/vol101/mono101-B02-B03.pdf NAP – Search Site		

System Used	Selected Key Reference(s) or Sources	Date	Additional References Identified
	(http://www.nap.edu/) NCI (http://www.cancer.gov) NCTR (http://www.fda.gov/AboutFDA/CentersOffices/OC/OfficeofScientificandMedicalPrograms/NCTR/default.htm) NIEHS http://www.niehs.nih.gov/ NICNAS (PEC only covered by eChemPortal) (http://www.nicnas.gov.au/industry/aics/search.asp) NIOSH (http://www.cdc.gov/niosh/topics/) NIOSHTIC 2 (http://www2a.cdc.gov/nioshtic-2/) NTP - RoC, status, results, and management reports 12th Report On Carcinogens: (http://ntp.niehs.nih.gov/?objectid=03C9AF75-E1BF-FF40-DBA9EC0928DF8B15) NTP Site Search: http://ntpsearch.niehs.nih.gov/texis/search/?query=arsenic&pr=ntp_web_entire_site_all&mu=Entire+NTP+Site OSHA (http://www.osha.gov/dts/chemicalsampling/toc/toc_chemsamp.html) RTECS http://www.ccohs.ca/search.html		

1

2

3. SELECTION OF STUDIES FOR HAZARD IDENTIFICATION

3.1. General Approach

The NRC (NRC, 2011) recommended that after studies are identified for review by utilizing a transparent search strategy, the next step is to summarize the details and findings of the most pertinent studies in evidence tables. The NRC suggested that such tables should provide a link to the references, and include details of the study population and methods and key findings. This approach provides for a systematic and concise presentation of the evidence. The NRC also recommended that the methods and findings should then be evaluated with a standardized approach. The approach that was outlined identified standard issues for the evaluation of epidemiological and experimental animal studies.

In response to the NRC recommendations, each study retained after the literature search and screen is evaluated for aspects of its design or conduct that could affect the interpretation of results and the overall contribution to the synthesis of evidence for determination of hazard potential. Much of the key information for conducting this evaluation can generally be found in the study's methods section and in how the study results are reported. Importantly, this evaluation does not consider study results or more specifically, the direction or magnitude of any reported effects. For example, standard issues for evaluation of experimental animal data identified by the NRC and adopted in this approach include consideration of the species and sex of animals studied, dosing information (dose spacing, dose duration, and route of exposure), endpoints considered, and the relevance of the endpoints to the human endpoints of concern.

To facilitate the evaluation outlined above, evidence tables are constructed that consistently summarize the important information from each study in a standardized tabular format as recommended by the NRC (NRC, 2011). In general, the evidence tables include all studies that inform the overall synthesis of evidence for hazard potential. At this stage, exclusion of studies may unnecessarily narrow subsequent analyses by eliminating information that might later prove useful. Premature exclusion might also give a false sense of the consistency of results across the database of studies by unknowingly reducing the diversity of study results. Thus, at this early stage of study evaluation the goal is to be inclusive.

Even at this early stage, however, a study can be excluded if flaws in its design or conduct are so great that the results would not be considered credible. Such study design flaws are discussed in a number of EPA's guidelines (see http://www.epa.gov/iris/backgrd.html) or

summarized in the draft Preamble to the IRIS Toxicological Review ("Preamble")[21]. Examples of these flaws include studies where impurities in the test chemical are so great as to prohibit attribution of the results to the chemical, or studies where concurrent or essential historical control information is lacking. Studies excluded because of fundamental flaws in their design or conduct are not included in evidence tables. Instead, text accompanying the evidence tables lists the reasons that studies were excluded.

The size of the database can influence both the type and number of evaluation criteria that are applied at this early stage. For example, if there are few studies on a health effect, additional evaluation criteria might not be needed, and thus the evidence tables may include all studies without severe flaws. Especially with smaller databases, it is important to consider all studies and not exclude studies unnecessarily. On the other hand, if there are many studies on a health effect (e.g., more than 20), additional criteria could facilitate a more efficient review of the database and help to focus on the more pertinent or stronger studies indicating the potential for hazard. These criteria could be specific to each type of study or a particular endpoint, and may consider factors such as those discussed in EPA's guidelines or summarized in the draft Preamble. Application of such additional criteria could result in initially setting aside some studies and not summarizing them in the evidence tables. Also, there may be situations in which the initial review of the available data will lead to a decision to focus on a particular set of health effects, and to exclude others from further evaluation. This situation could occur, for example, with a chemical with a large database with a few well-developed areas of research, but many other areas that consist of sparse data offering a very limited basis for drawing conclusions regarding hazard. In this case, EPA will focus on the more developed areas of research for hazard identification.

3.2. Selection of Primary Studies for Evidence Tables for HBCD

3.2.1. Epidemiologic Studies

The initial review of epidemiologic studies was conducted for those that were retained after the literature was manually screened for pertinence (title, abstract, and/or full text) (Figure 2-1; Primary Sources of Health Effects Data). Five epidemiologic studies examined associations between HBCD exposure and certain endocrine (including thyroid and reproductive hormone), neurobehavioral, and developmental outcomes. None of these studies had severe flaws that would compromise the credibility of their results. Because there are relatively few epidemiological studies of HBCD, these studies are all included in the preliminary evidence tables.

Two human studies were not summarized in the evidence tables. One study examined bone density as an outcome measure (Weiss et al., 2006), but no association with measures of HBCD

[21] See the draft Preamble in the Toxicological Review of Ammonia (revised external review draft) at http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=254524 or in the Toxicological Review of Trimethylbenzenes (revised external review draft) at http://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=254525.

exposure was observed and EPA is not further developing a review of this endpoint. A second study was a report of a human dermal patch test (McDonnell, 1972), a study design generally less pertinent for characterizing health hazards associated with chronic exposure. Nevertheless, these studies will still be considered as potential information sources during assessment development.

3.2.2. Experimental Animal Studies

An initial review was also performed for the experimental animal studies identified in the literature search and screen (Figure 2-1; Primary Sources of Health Effects Data). The HBCD experimental animal database is relatively small, and consists of studies designed to examine repeat-dose oral toxicity and specialized studies of reproductive and developmental toxicity, neurotoxicity and neurobehavioral toxicity, thyroid toxicity, and immunotoxicity. These studies are pertinent to evaluating the health effects of HBCD associated with human environmental exposure, and none had severe flaws that would compromise the credibility of their results. Because there are relatively few experimental animal toxicity studies of HBCD, these studies are all included in the preliminary evidence tables.

The HBCD experimental animal database also includes studies of acute toxicity and ocular and dermal irritation. As these short-duration studies are generally less pertinent for characterizing health hazards associated with chronic exposure, they are not summarized in the preliminary evidence tables. Nevertheless, these studies will still be evaluated as possible sources of toxicokinetic or mechanistic information during assessment development.

The experimental database contains genotoxicity and other mechanistic studies that will support the health assessment of HBCD (see Figure 2-1; Supporting Studies). Because mechanistic studies are numerous and their designs are highly heterogeneous, extracting study design information and results into evidence tables before identifying the health effects and potential modes of action (MOAs) and/or adverse outcome pathways (AOPs) that are scientifically plausible would be a resource intensive, yet potentially uninformative effort. Instead, for this group of studies, the preliminary materials provide a summary table of mechanistic studies (including general information on the test system/assays, measured parameters, and the possible health effect(s) to which each mechanistic study may relate) as a useful starting point for future analysis of support for possible MOAs/AOPs.

3.3. Preliminary Evidence Tables and Exposure-Response Arrays

Data from the primary studies identified by the approaches outlined above have been extracted and presented in evidence tables (Appendix A). The evidence tables present data from studies related to a specific outcome or endpoint of toxicity. At a minimum, the evidence tables include the relevant information for comparing key study characteristics such as study design, exposure metrics, and dose-response information. Evidence tables will serve as an additional method for presenting and evaluating the suitability of the data to inform hazard identification for

HBCD during the analysis of hazard potential and utility of the data for dose-response evaluation. The information in the preliminary evidence tables is also displayed graphically in preliminary exposure-response arrays. In these arrays, a significant effect (indicated by a filled circle) is based on statistical significance.

A compendium of genotoxicity and other mechanistic studies that will support the HBCD health assessment, with general information on the test system used and endpoints evaluated, is presented in a mechanistic study summary table (Appendix B).

The complete list of references considered in preparation of these materials can be found on the HERO website at http://hero.epa.gov/HBCD.

3.4. Study Characteristics That Will Be Considered in the Evaluation and Synthesis of the Primary Studies for HBCD

3.4.1. Epidemiologic Studies

Several considerations will be used in EPA's evaluation of the studies of human health effects of HBCD. The general considerations for evaluating issues relating to the study population, exposure, outcomes, confounding, and analysis are outlined in the draft Preamble. These, along with more specific issues pertaining to exposure and outcomes studied, are described below and in Table 3-1.

Study population

The general considerations for evaluating issues relating to the study population include adequate documentation of participant recruitment, including eligibility criteria, participation rates, missing data, loss to follow-up, and general demographic characteristics. This information is used to evaluate the potential for selection bias, as well as to facilitate comparison of results across different study populations. It is important to note that low participation rates, or even different participation rates between exposed and non-exposed or between cases and controls, are not evidence of selection bias. Rather, selection bias arises from a differential pattern of participation with respect to exposure *and* disease, e.g., if people with high exposure and the outcome of interest are more likely to participate than people with low exposure and the outcome.

The available epidemiological studies examined many different types of exposures (brominated flame retardants as well as other types of compounds) within the context of research on potential endocrine disruptors. Individuals typically do not have knowledge of their exposure to HBCD, and thus, knowledge of exposure or exposure level is unlikely to result in differential participation with respect to outcomes. However, EPA will consider the possibility that a particular concern about exposure to flame retardants would have motivated people to participate in a study or to continue participation throughout a follow-up period. EPA will also consider indirect ways in which a common factor could contribute both to HBCD exposure and to a specific outcome. In the

absence of evidence that any of these scenarios is at play, EPA will not consider selection bias attributed to these factors to be a likely limitation of a study.

Exposure measures

The general considerations for evaluating issues relating to exposure include characterization of exposure during the appropriate critical period for the outcomes under study, and use of appropriate ascertainment methods to classify individuals with regards to the exposure.

There are some exposure-related issues specific to HBCD. The major sources of (non-occupational) exposure to HBCD are indoor dust and diet. HBCD can be measured in biological samples; adipose tissue, serum and breast milk (which has a high proportion of lipids) are preferred over urine or saliva because of the accumulation of HBCD in fatty tissue and relatively long half-life of HBCD. The estimated half-life is likely on the order of weeks rather than days or hours (Geyer et al., 2004), and thus in general a single spot measurement is not considered a limitation for brominated flame retardants. However, HBCD levels could change more rapidly during pregnancy and lactation, due to mobilization of maternal fat stores (Aurell and Cramér, 1966); therefore EPA will consider these factors in evaluating the timing of sample collection in relation to the critical window of exposure, if known, for the outcome(s) under study. Studies of PBDEs in breast milk have shown relatively small variation in breast milk over time, with a 2–3% decrease in PBDE concentration per month (Daniels et al., 2010; Hooper et al., 2007); thus, measurements of HBCD levels in breast milk are likely to be a good surrogate for infant post-natal exposure, but less is known about the correlation with early periods of gestation. Measures of HBCD in dust are likely to correlate well with concentrations in biological samples. One study by Roosens et al. (2009) examined HBCD in serum and estimated HBCD ingestion from dust, and found a high correlation of 0.86 between these two measures. This result is similar to or stronger than correlations between other polybrominated flame retardant levels in dust and biomarker measures, with correlations ranging from 0.3–0.8 (Stapleton et al., 2012; Johnson et al., 2010; Wu et al., 2007).

Measurement of HBCD in serum raises an additional issue with respect to the potential need for adjustment for lipid levels, either through use of lipid adjusted serum concentrations, or inclusion of serum lipids as a covariate in multivariate analysis. Simulation studies indicate that the former approach (i.e., use of lipid adjusted concentrations) may lead to biased risk estimates (Schisterman et al., 2005). EPA will consider this potential bias in evaluating studies using lipid adjusted concentrations.

HBCD comprises three isomers; α-HBCD appears to bioaccumulate more readily compared to the other isomers, and may better reflect longer-term exposure. While some studies specify the isomer measured in biological samples (or state that all three were measured together and summed), others do not specify this information. HBCD levels from these studies may represent a 'total' HBCD concentration, which is likely to be dominated by α-HBCD unless a significant exposure

event had occurred in the very recent past. Thus, lack of specification of the isomer measured is unlikely to be a major limitation for epidemiology studies.

Another issue with HBCD measured in either biological tissue or environmental media is the limit of detection (LOD) for the assay. A high proportion of samples below the LOD can reduce the ability of the study to evaluate associations, and particularly exposure-response patterns.

EPA also considers the distribution of exposure in evaluating individual studies and comparing results among groups of studies. One consideration is the span of exposure levels (i.e., the contrast between "high" and "low"): a study with a very narrow span may not have sufficient variability to detect an effect that would be seen over a broader range. Another consideration is the absolute level of exposure: different effect estimates may be expected in studies examining different exposure levels.

Outcome measures

The general considerations for evaluating issues relating to outcomes include adequate duration of exposure and follow-up in order to evaluate the outcomes of interest, and use of appropriate ascertainment methods to classify individuals with regard to the outcome. The primary outcomes examined in the epidemiology studies are levels of the thyroid hormones (triiodothyronine, T3, and thyroxine, T4) and thyroid stimulating hormone (TSH) (or thyrotropin) produced by the pituitary, and neurobehavioral outcomes measured using validated instruments in infants and children.

The details of the laboratory procedures, including information on the basic methods, limit of detection, and coefficient of variation, are important considerations for the hormone assays. Thyroid hormones are generally measured in serum, although they may also be measured in whole dried blood spots, such as are collected from newborn infants in screening for congenital hypothyroidism as well as for genetic metabolic diseases such as phenylketonuria. Studies in older age groups have also shown a high correlation between thyroid hormone levels measured in dried blood spots and levels in serum (Hofman et al., 2003).

With respect to thyroid hormones, time of day and season are two potential sources of variability. For example, serum TSH measured shortly after midnight may be as much as twice as high as the value measured in late afternoon (Brabant et al., 1991; Weeke and Gundersen, 1978). The evidence with respect to seasonal variability is mixed (Plasqui et al., 2003; Maes et al., 1997; Nicolau et al., 1992; Simoni et al., 1990; Behall et al., 1984; Postmes et al., 1974) and this effect is likely to be smaller than that of time of day. The impact of these sources of variation will depend on whether they are also related to HBCD (i.e., do HBCD levels vary by time of day or season?). If this is the case, failure to address these factors in the design or analysis could result in confounding of the observed association, with the direction determined by the direction of the association between these factors and HBCD. If this is not the case, the lack of consideration of time of day or seasonality would result in greater variability in the hormone measures, and thus would result in more

imprecise (but not biased) estimates. EPA has not found evidence of a seasonal or diurnal variation in HBCD levels, and thus considers the latter scenario, i.e., lack of consideration of these factors leading to greater imprecision, rather than a biased effect estimate, to be more likely.

With respect to neurodevelopmental outcomes, a major consideration is the assessment tool(s) used by the study investigators; details of the assessment method, or references providing this information, should be provided. In addition, EPA also looks for discussion of (or reference to) validation studies and the appropriateness of the tool for evaluation in the specific study population (e.g., age range, language).

Confounding

The general considerations for evaluating issues relating to potential confounding include consideration of which factors may be potential confounders (i.e., those that are strongly related to both the exposure and the outcome under consideration), and if needed, control for these potential confounders in the study design or analysis. Adequacy of the measurement of confounders, and the potential for residual confounding, will also be considered.

Age and sex are considered important explanatory factors for the hormone measures, as well as for the neuropsychological and neurobehavioral outcomes, even in the absence of strong associations with HBCD (Rawn et al., 2014). A measure of socioeconomic status (e.g., parental education level) is also typically used in studies of cognition and behavioral outcomes, although associations between HBCD and socioeconomic status have not been established.

Analysis

The general considerations for evaluating issues relating to analysis are outlined in the draft Preamble. These include adequate documentation of analytic approach to interpret study results, consideration of sample size and statistical power, and use of appropriate methods for the study design.

As noted above, a major analytic consideration is how lack of variability in the exposure and/or the outcome(s) is addressed—for example, as may occur if many HBCD measurements fall below the LOD. The study should describe the distribution of HBCD exposure and outcome(s) in the study population (for both the study and comparison groups), and if needed, use appropriate analytic techniques to address lack of variability, or unusual or skewed distributions.

Table 3-1. General and outcome-specific considerations for HBCD human study evaluation

General considerations	
Study population	- Study population and setting: geographic area, site, time period, age and sex distribution, other details as needed (may include race/ethnicity, socioeconomic status) - Recruitment process; exclusion and inclusion criteria, knowledge of study hypothesis, knowledge of exposure and outcome - Participation rates: Total eligible, participation at each stage and for final analysis group and denominators used to make these calculations - Length of follow-up, loss to follow-up - Comparability: Participant characteristic data by group, data on non-participants
Exposure	- Specific HBCD isomer(s) measured - Limit of detection (LOD) or level of quantitation (LOQ) - Exposure distribution (e.g., central tendency, range), proportion < LOD
Analysis	- Consideration of skewness of exposure and outcome measures - Consideration of values below LOD or LOQ - Consideration of lipids (for serum or breast milk samples) adjustment - Presentation of quantitative results, rather than statement regarding presence or absence of statistical significance
Outcome-specific considerations	
Neuropsychological and neurobehavioral **Measures**	- Standardized assessment tool, validation studies for specific study population (e.g., age group, geographic location) - Blinding of assessor to exposure
Consideration of confounding	- Age, sex, socioeconomic status
Thyroid **Measures**	- Assay used and evidence from validation studies, if available - Sensitivity/detection limits, coefficient of variation; number of samples below LOD - Biological sample used (e.g., serum, dried whole blood spots) - Time of day and season when samples for thyroid hormone (and TSH) collected
Consideration of confounding	- Age, sex

3.4.2. Experimental Animal Studies

Beyond the initial methodological screening described above in Section 3.2.2, methodological aspects of a study's design and conduct will be considered again in the overall evaluation and synthesis of the pertinent data that will be developed for each health effect. Some general questions that will be considered in evaluating experimental animal studies are presented in Table 3-2. These questions are, for the most part, broadly applicable to all experimental studies.

Table 3-2. Questions and relevant experimental information for evaluation of experimental animal studies

Methodological feature	Question(s) considered	Examples of relevant information extracted
Test animal	Based on the endpoint(s) in question, are concerns raised regarding the suitability of the species, strain, or sex of the test animals on study?	Test animal species, strain, sex
Experimental setup	Are the timing, frequency and duration of exposure, as well as animal age and experimental group allocation procedures/ group size for each endpoint evaluation, appropriate for the assessed endpoint(s)?	Age/lifestage of test animals at exposure and all endpoint testing timepoints Timing and periodicity of exposure and endpoint evaluations; duration of exposure Experimental group allocation procedures and sample size for each experimental group (e.g., animals; litters; dams) at each endpoint evaluation
Exposure	Are the exposure conditions and controls informative and reliable for the endpoint(s) in question, and are they sufficiently specific to the compound of interest?	Test article composition, stability, and vehicle control Exposure administration techniques (e.g., route; chamber type) and related controls
Endpoint evaluation procedures	Do the procedures used to evaluate the endpoint(s) in question conform to established protocols, or are they biologically sound? Are they sensitive for examination of the outcome(s) of interest?	Specific methods for assessing the effect(s) of exposure, including related details (e.g., biological matrix or specific region of tissue/organ evaluated) Endpoint evaluation controls, including those put in place to minimize evaluator bias
Outcomes and data reporting	Were data reported for all pre-specified endpoint(s) and study groups, or were any data excluded from presentation/ analyses?	Data presentation for endpoint(s) of interest

Note: "Outcome" refers to findings from an evaluation (e.g., steatosis), whereas "endpoint" refers to the evaluation itself (e.g., liver histopathology).

Evaluation of some specific methodological features identified in Table 3-2, such as exposure, is likely to be relatively independent of outcome. Other methodological features, in particular those related to experimental setup and endpoint evaluation procedures, are generally outcome specific (e.g., reproductive and developmental toxicity). Some specific aspects of study methodology that will be considered in the evaluation and synthesis of the HBCD literature are described below.

Exposure

Commercial HBCD consists of three primary isomers (α, β, and γ). Because these isomers display different toxicokinetic properties, the isomeric composition of the test material could influence study results. Accordingly, the isomeric composition tested in each study will be considered in the development of the synthesis. Information on purity of the commercial mixtures may be important as well. Information on the test material as reported by study authors for those experimental animal studies included in preliminary evidence tables is summarized in Appendix B (Table A-8).

The majority of studies administered HBCD in the diet. Because HBCD is semivolatile and can partition into the atmosphere when exposed to air, documentation of stability of the test material in the diet will be a consideration.

Outcome-specific Considerations

In general, experimental animal studies will be compared against traditional assay formats (e.g., those used in guideline studies), with deviations from the protocol evaluated in light of how the deviations could alter interpretation of the outcome in question. A number of the HBCD studies applied study protocols to examine effects of HBCD on the thyroid, nervous system, reproduction, development, and immune system.

<u>Thyroid Endpoints</u>

The HBCD experimental animal database includes several studies of the potential effects of HBCD on the thyroid, and in particular thyroid hormone level testing. Specific Agency guidelines on testing and evaluation of thyroid endpoints are not available. Some considerations for evaluating studies of thyroid endpoints include the following:

- Radioimmunoassays (RIA) are generally the standard for measuring thyroid hormones in rodent studies. Results from ELIZA assays should be interpreted cautiously; reported detection limits should be based on within-laboratory calibrations and not on assay specs. [The specific assay used in the studies that measured thyroid hormones are reported in the preliminary evidence tables.]
- Hormones should be sampled at the same time of day because of fluctuations in T3 and T4 levels throughout the day in rats.
- Whether both male and female animals were tested because of possible gender differences (e.g., differences associated with maturation of reproductive hormone systems and cyclicity in females).
- Hormone testing protocols will be evaluated further for other experimental setup features and endpoint evaluation procedures, including sensitivity/detection limit calibrations for each assay, validation of assays using heterologous antibodies, extent of outcomes below the limit of detection (LOD), and minimization of nontreatment-related influences on

hormone levels (e.g., stress-induced alterations, thyroid hormone suppression from anesthesia), and inclusion of positive control treatment with expected serum thyroid hormone pattern (e.g., methimazole or propylthiouracil).

Neurological and Neurobehavioral Endpoints

The HBCD experimental database includes functional observational batteries (FOB) in adult rats, and protocols to examine motor function-related behaviors and cognition (memory assessed using water maze) in rat pups in a multigenerational study, locomotor activity in mice pups, and electrophysiology in rats following developmental exposure. In general, assays used in studies will be compared to traditional assay formats for evaluating these specific neurotoxicity endpoints.

EPA's Guidelines for Neurotoxicity Risk Assessment (U.S. EPA, 1998a) outline important aspects of study design that should be considered when assessing neurotoxicity endpoints; as applicable, these guidelines will be used for characterizing and interpreting assay results from neurotoxicity studies. For those studies of neurotoxicity endpoints evaluated in experimental animals exposed during development, additional considerations provided in EPA's Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991) are applicable. In addition, the following considerations regarding specific tests employed in the HBCD database will be incorporated:

- For all behavioral assays, it is desirable for the investigators recording the responses to be blinded as to the treatment of the test animals.
- Tests of motor activity should be of sufficient duration (e.g., ≥20 minutes), and should be evaluated in the absence of evidence of systemic toxicity, as this may cause misinterpretation due to nonneurotoxic effects. While tests of shorter duration may still be useful, consideration should be paid to the involvement of behaviors other than motor function. For non-developmental evaluations of motor function (e.g., coordination and dexterity), it is desirable that the results be presented as continuous rather than (or in addition to) dichotomized data, as it is problematic to arbitrarily define a response as a "success" or "failure," particularly without first establishing a baseline across a large number of animals with the same phenotype, housing, and test conditions.
- FOBs typically represent a standardized series of tests evaluating various domains of nervous system function within a short time period (e.g., 10 minutes). While most studies use a validated FOB design, application of the FOB can vary across laboratories, which can introduce additional uncertainty. For example, an FOB should take care to consider and account for the order of testing, as order effects in these batteries can introduce nonspecific effects.
- Measurements of memory and learning should be separated from other changes in behavior that do not involve cognitive processes (e.g., motor function). Specifically regarding water maze tests, the temperature of the water bath, platform and pool size, and visual cues (including the investigator) necessary for accomplishing the task should be controlled for

and appropriate to the test animal species and age under investigation.

Reproductive and developmental endpoints

The HBCD database includes 1- and 2-generation reproductive toxicity studies, a developmental study, and other repeat-dose studies that examined reproductive organs. EPA's Guidelines for Reproductive Toxicity Risk Assessment (U.S. EPA, 1996) detail study design parameters that are of particular importance in reproductive toxicity studies. These factors include duration of dosing, length of mating period and number of males and females mated; type of test (single versus multigeneration studies); and endpoints evaluated. Test guidelines for the conduct of single- and multigeneration reproduction protocols that have been published by EPA and OECD will be utilized in evaluation of the reproductive and developmental toxicity database for HBCD (U.S. EPA, 1996, 1985; Galbraith et al., 1983; OECD, 1983).

Likewise, EPA's Guidelines for Developmental Toxicity Risk Assessment (U.S. EPA, 1991) detail study design parameters that are of particular importance in developmental toxicity studies. Evaluation of developmental endpoints includes studies that typically involve exposure of pregnant animals during critical windows of organogenesis, evaluation of maternal toxicity throughout pregnancy, and examination of dams and uterine contents (U.S. EPA, 1991). Developmental toxicity studies also may evaluate exposures of one to a few days to investigate critical windows of development. Endpoints typically evaluated in developmental toxicity studies include assessment of maternal toxicity, altered survival and growth, morphological development, and functional deficits. A particular consideration in developmental toxicity studies is the selection of a high dose that produces minimal maternal or adult toxicity (i.e., a level that at the least produces marginal but significantly reduced body weight, reduced weight gain, or specific organ toxicity, and at the most produces no more than 10% mortality). At doses that cause excessive maternal toxicity (that is, significantly greater than the minimal toxic level), information on developmental effects may be difficult to interpret and of limited value.

Immune endpoints

The HBCD database includes limited testing of immunotoxic potential, largely focused on cell counting, and functional immune assays. In general, functional assays will be weighed more heavily than observational endpoints such as cell counts and organ weights. Immunotoxicity testing guidelines will be used to evaluate adherence to established protocols and to incorporate current guidance practices for assessing immune endpoings, including the following:

- WHO/International Programme on Chemical Safety (IPCS) Harmonization Project Document No. 10, Guidance for Immunotoxicity Risk Assessment for Chemicals (WHO, 2012) (available at http://www.inchem.org/documents/harmproj/harmproj/harmproj10.pdf).
- WHO/International Programme on Chemical Safety (IPCS) Environmental Health Criteria

180: Principles and Methods for Assessing Direct Immunotoxicity Associated with Exposure to Chemicals (WHO, 1996) (available at http://www.inchem.org/documents/ehc/ehc/ehc180.htm).

- U.S. EPA Health Effects Test Guidelines, OPPTS 870.7800, Immunotoxicity (U.S. EPA, 1998b) (available at http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2009-0156-0049).
- OECD Test Guidelines 443 (Extended One-Generation Reproductive Toxicity Study test guideline), 407 (Repeated Dose 28-day Oral Toxicity Study in Rodents test guideline), 408 (Repeat Dose 90-day Oral Toxicity in Rodents test guideline), and 413 (Subchronic Inhalation Toxicity: 90- Day Study test guideline), which include endpoints that may give an indication of immunological effects or, in the case of Test Guideline 443, developmental immunotoxicity. (available at http://www.oecd.org/env/ehs/testing/oecdguidelinesforthetestingofchemicals.htm).

A full evaluation of all pertinent studies will be performed as part of the critical review and synthesis of evidence for hazard identification for each of the health endpoints identified in the evidence tables (Appendix A).

4. REFERENCE LIST

Al-Mousa, F; Michelangeli, F. (2012). Some commonly used brominated flame retardants cause Ca2+-ATPase inhibition, beta-amyloid peptide release and apoptosis in SH-SY5Y neuronal cells. PLoS ONE 7: e33059. http://dx.doi.org/10.1371/journal.pone.0033059

An, J; Zou, W; Chen, C; Zhong, FY; Yu, QZ; Wang, QJ. (2013). The cytological effects of HBCDs on human hepatocyte L02 and the potential molecular mechanism. J Environ Sci Health A Tox Hazard Subst Environ Eng 48: 1333-1342. http://dx.doi.org/10.1080/10934529.2013.781875

Aniagu, SO; Williams, TD; Allen, Y; Katsiadaki, I; Chipman, JK. (2008). Global genomic methylation levels in the liver and gonads of the three-spine stickleback (Gasterosteus aculeatus) after exposure to hexabromocyclododecane and 17-beta oestradiol. Environ Int 34: 310-317. http://dx.doi.org/10.1016/j.envint.2007.03.009

Aurell, M; Cramér, K. (1966). Serum lipids and lipoproteins in human pregnancy. Clin Chim Acta 13: 278-284. http://dx.doi.org/10.1016/0009-8981(66)90206-3

BASF. (1990). Hexabromocyclododecane 28-day feeding trials with rats with test data and cover letter. (86900000274). Washington, DC: U.S. Environmental Protection Agency.

BASF. (2000). Cytogenetic study in vivo with of hexabromocyclododecane in the mouse micronucleus test after two intraperitoneal administrations. (Project No. 26MO100/004018). Ludwigshafen, Germany: BASF Aktiengesellschaft.

Bastos Sales, L; Kamstra, JH; Cenijn, PH; van Rijt, LS; Hamers, T; Legler, J. (In Press) Effects of endocrine disrupting chemicals on in vitro global DNA methylation and adipocyte differentiation. Toxicol In Vitro. http://dx.doi.org/10.1016/j.tiv.2013.04.005

Behall, KM; Scholfield, DJ; Hallfrisch, JG; Kelsay, JL; Reiser, S. (1984). Seasonal variation in plasma glucose and hormone levels in adult men and women. Am J Clin Nutr 40: 1352-1356.

Brabant, G; Prank, K; Hoang-Vu, C; Hesch, RD; von Zur Mühlen, A. (1991). Hypothalamic regulation of pulsatile thyrotopin secretion. J Clin Endocrinol Metab 72: 145-150. http://dx.doi.org/10.1210/jcem-72-1-145

Cantón, RF; Sanderson, JT; Nijmeijer, S; Bergman, A; Letcher, RJ; van den Berg, M. (2006). In vitro effects of brominated flame retardants and metabolites on CYP17 catalytic activity: a novel mechanism of action? Toxicol Appl Pharmacol 216: 274-281. http://dx.doi.org/10.1016/j.taap.2006.05.007

Crump, D; Chiu, S; Egloff, C; Kennedy, SW. (2008). Effects of hexabromocyclododecane and polybrominated diphenyl ethers on mRNA expression in chicken (Gallus domesticus) hepatocytes. Toxicol Sci 106: 479-487. http://dx.doi.org/10.1093/toxsci/kfn196

Crump, D; Egloff, C; Chiu, S; Letcher, RJ; Chu, S; Kennedy, SW. (2010). Pipping success, isomer-specific accumulation, and hepatic mRNA expression in chicken embryos exposed to HBCD. Toxicol Sci 115: 492-500. http://dx.doi.org/10.1093/toxsci/kfq068

Daniels, JL; Pan, IJ, en; Jones, R; Anderson, S; Patterson, DG, Jr; Needham, LL; Sjodin, A. (2010). Individual Characteristics Associated with PBDE Levels in US Human Milk Samples. Environ Health Perspect 118: 155-160. http://dx.doi.org/10.1289/ehp.0900759

Deng, J; Yu, L; Liu, C; Yu, K; Shi, X; Yeung, LW; Lam, PK; Wu, RS; Zhou, B. (2009). Hexabromocyclododecane-induced developmental toxicity and apoptosis in zebrafish embryos. Aquat Toxicol 93: 29-36. http://dx.doi.org/10.1016/j.aquatox.2009.03.001

Dingemans, MM; Heusinkveld, HJ; de Groot, A; Bergman, A; van den Berg, M; Westerink, RH. (2009). Hexabromocyclododecane inhibits depolarization-induced increase in intracellular calcium levels and neurotransmitter release in PC12 cells. Toxicol Sci 107: 490-497. http://dx.doi.org/10.1093/toxsci/kfn249

Du, M; Zhang, D; Yan, C; Zhang, X. (2012). Developmental toxicity evaluation of three hexabromocyclododecane diastereoisomers on zebrafish embryos. Aquat Toxicol 112-113: 1-10. http://dx.doi.org/10.1016/j.aquatox.2012.01.013

Eggesbø, M; Thomsen, C; Jørgensen, JV; Becher, G; Odland, JØ; Longnecker, MP. (2011). Associations between brominated flame retardants in human milk and thyroid-stimulating hormone (TSH) in neonates. Environ Res 111: 737-743. http://dx.doi.org/10.1016/j.envres.2011.05.004

Ema, M; Fujii, S; Hirata-Koizumi, M; Matsumoto, M. (2008). Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats. Reprod Toxicol 25: 335-351. http://dx.doi.org/10.1016/j.reprotox.2007.12.004

Eriksson, P; Fischer, C; Wallin, M; Jakobsson, E; Fredriksson, A. (2006). Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD). Environ Toxicol Pharmacol 21: 317-322. http://dx.doi.org/10.1016/j.etap.2005.10.001

Ethyl Corporation. (1990). Genetic toxicology rat hepatocyte primary culture/DNA repair test on hexabromocyclododecane with cover letter dated 030890. Baton Rouge, LA. http://www.ntis.gov/search/product.aspx?ABBR=OTS0522234

Fa, S; Pogrmic-Majkic, K; Dakic, V; Kaisarevic, S; Hrubik, J; Andric, N; Stojilkovic, SS; Kovacevic, R. (2013). Acute effects of hexabromocyclododecane on Leydig cell cyclic nucleotide signaling and steroidogenesis in vitro. Toxicol Lett 218: 81-90. http://dx.doi.org/10.1016/j.toxlet.2013.01.009

Fernandez Canton, R; Sanderson, T; Nijmeijer, S; Bergman, A; Van Den Berg, M. (2005). In vitro effects of brominated flame retardants on the adrenocortical enzyme CYP17. A novel endocrine mechanism of action? [Abstract]. Toxicologist 84: 356.

Galbraith, WM; Voytek, P; Ryon, MS. (1983). Assessment of risks to human reproduction and development of the human conceptus from exposure to environmental substances. In Advances in Modern Environmental Toxicology. Princeton, NJ: Princeton Scientific Publishing.

Geyer, HJ; Schramm, K, -W; Darnerud, PO; Aune, M; Feicht, EA; Fried, KW; Henkelmann, B; Lenoir, D; Schmid, P; McDonald, TA. (2004). Terminal elimination half-lives of the brominated flame retardants TBBPA, HBCD, and lower brominated PBDEs in humans. Organohalogen Compounds 66: 3820-3825.

Harju, M; Hamers, T; Kamstra, JH; Sonneveld, E; Boon, JP; Tysklind, M; Andersson, PL. (2007). Quantitative structure-activity relationship modeling on in vitro endocrine effects and metabolic stability involving 26 selected brominated flame retardants. Environ Toxicol Chem 26: 816-826. http://dx.doi.org/10.1897/06-308R.1

Helleday, T; Tuominen, KL; Bergman, A; Jenssen, D. (1999). Brominated flame retardants induce intragenic recombination in mammalian cells. Mutat Res 439: 137-147.

Hinkson, NC; Whalen, MM. (2009). Hexabromocyclododecane decreases the lytic function and ATP levels of human natural killer cells. J Appl Toxicol 29: 656-661. http://dx.doi.org/10.1002/jat.1453

Hinkson, NC; Whalen, MM. (2010). Hexabromocyclododecane decreases tumor-cell-binding capacity and cell-surface protein expression of human natural killer cells. J Appl Toxicol 30: 302-309. http://dx.doi.org/10.1002/jat.1495

Hofman, LF; Foley, TP; Henry, JJ; Naylor, EW. (2003). Assays for thyroid-stimulating hormone using dried blood spotted filter paper specimens to screen for hypothyroidism in older children and adults. J Med Screen 10: 5-10. http://dx.doi.org/10.1258/096914103321610734

Hooper, K; She, J; Sharp, M; Chow, J; Jewell, N; Gephart, R; Holden, A. (2007). Depuration of polybrominated diphenyl ethers (PBDEs) and polychlorinated biphenyls (PCBs) in breast milk from California first-time mothers (primiparae). Environ Health Perspect 115: 1271-1275. http://dx.doi.org/10.1289/ehp.10166

Hu, J; Liang, Y; Chen, M; Wang, X. (2009a). Assessing the toxicity of TBBPA and HBCD by zebrafish embryo toxicity assay and biomarker analysis. Environ Toxicol 24: 334-342. http://dx.doi.org/10.1002/tox.20436

Hu, X; Hu, D; Xu, Y. (2009b). Effects of tetrabrominated diphenyl ether and hexabromocyclododecanes in single and complex exposure to hepatoma HepG2 cells. Environ Toxicol Pharmacol 27: 327-337. http://dx.doi.org/10.1016/j.etap.2008.11.014

Huntingdon Research Centre. (1990). Ames metabolic activation test to assess the potential mutagenic effect of und no. 49 with cover letter dated 031290 [TSCA Submission]. (86900000385). Wyandotte, MI: BASF Corporation. http://www.ntis.gov/search/product.aspx?ABBR=OTS0522948

Ibhazehiebo, K; Iwasaki, T; Shimokawa, N; Koibuchi, N. (2011). 1,2,5,6,9,10-αHexabromocyclododecane (HBCD) impairs thyroid hormone-induced dendrite arborization of Purkinje cells and suppresses thyroid hormone receptor-mediated transcription. Cerebellum 10: 22-31. http://dx.doi.org/10.1007/s12311-010-0218-1

Industrial Bio-Test Laboratories, Inc., . (1990). Mutagenicity of two lots of FM-100 lot 53 and residue of lot 3322 in the absence and presence of metabolic activation with test data and cover letter [TSCA Submission]. (86900000267). West Lafayette, IN: Great Lakes Chemical Corporation. http://www.ntis.gov/search/product.aspx?ABBR=OTS0523259

Johnson, PI; Stapleton, HM; Mukherjee, B; Hauser, R; Meeker, JD. (2013). Associations between brominated flame retardants in house dust and hormone levels in men. Sci Total Environ 445-446: 177-184. http://dx.doi.org/10.1016/j.scitotenv.2012.12.017

Johnson, PI; Stapleton, HM; Sjodin, A; Meeker, JD. (2010). Relationships between polybrominated diphenyl ether concentrations in house dust and serum. Environ Sci Technol 44: 5627-5632. http://dx.doi.org/10.1021/es100697q

Kang, NH; Hwang, KA; Kim, TH; Hyun, SH; Jeung, EB; Choi, KC. (2012). Induced growth of BG-1 ovarian cancer cells by 17β-estradiol or various endocrine disrupting chemicals was reversed by resveratrol via downregulation of cell cycle progression. Molecular Medicine Reports 6: 151-156. http://dx.doi.org/10.3892/mmr.2012.887

Kiciński, M; Viaene, MK; Den Hond, E; Schoeters, G; Covaci, A; Dirtu, AC; Nelen, V; Bruckers, L; Croes, K; Sioen, I; Baeyens, W; Van Larebeke, N; Nawrot, TS. (2012). Neurobehavioral function and low-level exposure to brominated flame retardants in adolescents: A cross-sectional study. Environ Health 11: 86. http://dx.doi.org/10.1186/1476-069X-11-86

Kling, P; Förlin, L. (2009). Proteomic studies in zebrafish liver cells exposed to the brominated flame retardants HBCD and TBBPA. Ecotoxicol Environ Saf 72: 1985-1993. http://dx.doi.org/10.1016/j.ecoenv.2009.04.018

Koike, E; Yanagisawa, R; Takigami, H; Takano, H. (2012). Brominated flame retardants stimulate mouse immune cells in vitro. J Appl Toxicol 33: 1451-1459. http://dx.doi.org/10.1002/jat.2809

Kurakawa, Y; Inoue, T; Uchida, Y; Momma, J. (1984). Carcinogenesis test of flame retarder hexabromocyclododecane in mice (unpublished, translated into English). Available online

Lilienthal, H; van Der Ven, L, eo; Hack, A; Roth-Harer, A; Piersma, A; Vos, J. (2009a). Neurobehavioral Effects in Relation to Endocrine Alterations Caused by Exposure to Brominated Flame Retardants in Rats-Comparison to Polychlorinated Biphenyls. Hum Ecol Risk Assess 15: 76-86. http://dx.doi.org/10.1080/10807030802615253

Lilienthal, H; van der Ven, LT; Piersma, AH; Vos, JG. (2009b). Effects of the brominated flame retardant hexabromocyclododecane (HBCD) on dopamine-dependent behavior and brainstem auditory evoked potentials in a one-generation reproduction study in Wistar rats. Toxicol Lett 185: 63-72. http://dx.doi.org/10.1016/j.toxlet.2008.12.002

Litton Bionetics. (1990). Mutagenicity evaluation of 421-32B (final report) with test data and cover letter [TSCA Submission]. (86900000265). West Lafayette, IN: Great Lakes Chemical Corporation. http://www.ntis.gov/search/product.aspx?ABBR=OTS0523257

Maes, M; Mommen, K; Hendrickx, D; Peeters, D; D'hondt, P; Ranjan, R; De Meyer, F; Scharpe, S. (1997). Components of biological variation, including seasonality, in blood concentrations of TSH, TT3, FT4, PRL, cortisol and testosterone in healthy volunteers. Clin Endocrinol 46: 587-598. http://dx.doi.org/10.1046/j.1365-2265.1997.1881002.x

Maranghi, F; Tassinari, R; Moracci, G; Altieri, I; Rasinger, JD; Carroll, TS; Hogstrand, C; Lundebye, AK; Mantovani, A. (2013). Dietary exposure of juvenile female mice to polyhalogenated seafood contaminants (HBCD, BDE-47, PCB-153, TCDD): comparative assessment of effects in potential target tissues. Food Chem Toxicol 56: 443-449. http://dx.doi.org/10.1016/j.fct.2013.02.056

Mariussen, E; Fonnum, F. (2003). The effect of brominated flame retardants on neurotransmitter uptake into rat brain synaptosomes and vesicles. Neurochem Int 43: 533-542. http://dx.doi.org/10.1016/S0197-0186(03)00044-5

McDonnell, ME. (1972). Human patch test - 20 subjects. (Haskell Laboratory Report 185-72). Haskell Laboratory for Toxicology and Industrial Medicine, E.I. du Pont de Nemours and Company.

Meijer, L; Martijn, A; Melessen, J; Brouwer, A; Weiss, J; de Jong, FH; Sauer, PJ. (2012). Influence of prenatal organohalogen levels on infant male sexual development: sex hormone levels, testes volume and penile length. Hum Reprod 27: 867-872. http://dx.doi.org/10.1093/humrep/der426

Microbiological Associates. (1996). Hexabromocyclododecane (HBCD): Chromosome aberrations in human peripheral blood lymphocytes with cover letter dated 12/12/1996 [TSCA Submission]. (86970000358). Arlington, VA: Chemical Manufacturers Association. http://www.ntis.gov/search/product.aspx?ABBR=OTS0573552

Nicolau, GY; Haus, E; Plîngă, L; Dumitriu, L; Lakatua, D; Popescu, M; Ungureanu, E; Sackett-Lundeen, L; Petrescu, E. (1992). Chronobiology of pituitary-thyroid functions. 30: 125-148.

NRC (National Research Council). (2009). Science and decisions: Advancing risk assessment. Washington, DC: National Academies Press. http://www.nap.edu/catalog/12209.html

NRC (National Research Council). (2011). Review of the Environmental Protection Agency's draft IRIS assessment of formaldehyde. Washington, DC: National Academies Press. http://www.nap.edu/catalog/13142.html

OECD (Organisation for Economic Co-operation and Development). (1983). First addendum to OECD guidelines for testing chemicals. Section 4, no. 415: One-Generation reproduction toxicity. Paris.

Ogaswara, S; Fukushi, A; Midorikawa, Y. (1983). Report on acute toxicity test of Pyroguard SR-103 in rats [unpublished]. Ogaswara, S; Fukushi, A; Midorikawa, Y.

Palace, V; Park, B; Pleskach, K; Gemmill, B; Tomy, G. (2010). Altered thyroxine metabolism in rainbow trout (Oncorhynchus mykiss) exposed to hexabromocyclododecane (HBCD). Chemosphere 80: 165-169. http://dx.doi.org/10.1016/j.chemosphere.2010.03.016

Palace, VP; Pleskach, K; Halldorson, T; Danell, R; Wautier, K; Evans, B; Alaee, M; Marvin, C; Tomy, GT. (2008). Biotransformation enzymes and thyroid axis disruption in juvenile rainbow trout (Oncorhynchus mykiss) exposed to hexabromocyclododecane diastereoisomers. Environ Sci Technol 42: 1967-1972. http://dx.doi.org/10.1021/es702565h

Park, MA; Hwang, KA; Lee, HR; Yi, BR; Jeung, EB; Choi, KC. (2012). Cell growth of BG-1 ovarian cancer cells is promoted by di-n-butyl phthalate and hexabromocyclododecane via upregulation of the cyclin D and cyclin-dependent kinase-4 genes. Molecular Medicine Reports 5: 761-766. http://dx.doi.org/10.3892/mmr.2011.712

Pharmakologisches Inst (Pharmakologisches Institute). (1990a). Ames Test with Hexabromides with Cover Letter dated 031290. (86900000379). Washington, DC: U.S. Environmental Protection Agency.

Pharmakologisches Inst (Pharmakologisches Institute). (1990b). Hexabromocyclododecane: 90-day feeding trials with rats with attachments and cover letter dated 031290. (86900000380). Washington, DC: U.S. Environmental Protection Agency.

Plasqui, G; Kester, AD; Westerterp, KR. (2003). Seasonal variation in sleeping metabolic rate, thyroid activity, and leptin. Am J Physiol Endocrinol Metab 285: E338-E343. http://dx.doi.org/10.1152/ajpendo.00488.2002

Postmes, TJ; Van Hout, JC; Saat, G; Willems, P; Coenegracht, J. (1974). A radioimmunoassay study and comparison of seasonal variation in plasma triiodothyronine and thyroxine concentrations in normal healthy persons. Clin Chim Acta 50: 189-195. http://dx.doi.org/10.1016/0009-8981(74)90366-0

Rawn, DF; Ryan, JJ; Sadler, AR; Sun, WF; Weber, D; Laffey, P; Haines, D; Macey, K; Van Oostdam, J. (2014). Brominated flame retardant concentrations in sera from the Canadian Health Measures Survey (CHMS) from 2007 to 2009. Environ Int 63: 26-34. http://dx.doi.org/10.1016/j.envint.2013.10.012

Reistad, T; Fonnum, F; Mariussen, E. (2006). Neurotoxicity of the pentabrominated diphenyl ether mixture, DE-71, and hexabromocyclododecane (HBCD) in rat cerebellar granule cells in vitro. Arch Toxicol 80: 785-796. http://dx.doi.org/10.1007/s00204-006-0099-8

Ronisz, D; Finne, EF; Karlsson, H; Förlin, L. (2004). Effects of the brominated flame retardants hexabromocyclododecane (HBCDD), and tetrabromobisphenol A (TBBPA), on hepatic enzymes and other biomarkers in juvenile rainbow trout and feral eelpout. Aquat Toxicol 69: 229-245. http://dx.doi.org/10.1016/j.aquatox.2004.05.007

Roosens, L; Abdallah, MA; Harrad, S; Neels, H; Covaci, A. (2009). Exposure to hexabromocyclododecanes (HBCDs) via dust ingestion, but not diet, correlates with concentrations in human serum: preliminary results. Environ Health Perspect 117: 1707-1712. http://dx.doi.org/10.1289/ehp.0900869

Roze, E; Meijer, L; Bakker, A; Van Braeckel, KN; Sauer, PJ; Bos, AF. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age. Environ Health Perspect 117: 1953-1958. http://dx.doi.org/10.1289/ehp.0901015

Saegusa, Y; Fujimoto, H; Woo, GH; Inoue, K; Takahashi, M; Mitsumori, K; Hirose, M; Nishikawa, A; Shibutani, M. (2009). Developmental toxicity of brominated flame retardants, tetrabromobisphenol A and 1,2,5,6,9,10-hexabromocyclododecane, in rat offspring after maternal exposure from mid-gestation through lactation. Reprod Toxicol 28: 456-467. http://dx.doi.org/10.1016/j.reprotox.2009.06.011

Saegusa, Y; Fujimoto, H; Woo, GH; Ohishi, T; Wang, L; Mitsumori, K; Nishikawa, A; Shibutani, M. (2012). Transient aberration of neuronal development in the hippocampal dentate gyrus after developmental exposure to brominated flame retardants in rats. Arch Toxicol 86: 1431-1442. http://dx.doi.org/10.1007/s00204-012-0824-4

Sakai, H; Kim, EY; Petrov, EA; Tanabe, S; Iwata, H. (2009). Transactivation potencies of Baikal seal constitutive active/androstane receptor by persistent organic pollutants and brominated flame retardants. Environ Sci Technol 43: 6391-6397. http://dx.doi.org/10.1021/es901120r

Schisterman, EF; Whitcomb, BW; Louis, GM; Louis, TA. (2005). Lipid adjustment in the analysis of environmental contaminants and human health risks. Environ Health Perspect 113: 853-857. http://dx.doi.org/10.1289/ehp.7640

Schriks, M; Roessig, JM; Murk, AJ; Furlow, JD. (2007). Thyroid hormone receptor isoform selectivity of thyroid hormone disrupting compounds quantified with an in vitro reporter gene assay. Environ Toxicol Pharmacol 23: 302-307. http://dx.doi.org/10.1016/j.etap.2006.11.007

Schriks, M; Vrabie, CM; Gutleb, AC; Faassen, EJ; Rietjens, IM; Murk, AJ. (2006a). T-screen to quantify functional potentiating, antagonistic and thyroid hormone-like activities of poly halogenated aromatic hydrocarbons (PHAHs). Toxicol In Vitro 20: 490-498. http://dx.doi.org/10.1016/j.tiv.2005.09.001

Schriks, M; Zvinavashe, E; Furlow, JD; Murk, AJ. (2006b). Disruption of thyroid hormone-mediated Xenopus laevis tadpole tail tip regression by hexabromocyclododecane (HBCD) and 2,2',3,3',4,4',5,5',6-nona brominated diphenyl ether (BDE206). Chemosphere 65: 1904-1908. http://dx.doi.org/10.1016/j.chemosphere.2006.07.077

Simoni, M; Velardo, A; Montanini, V; Faustini Fustini, M; Seghedoni, S; Marrama, P. (1990). Circannual rhythm of plasma thyrotropin in middle-aged and old euthyroid subjects. Horm Res 33: 184-189.

SRI International. (1990). In vitro microbiological mutagenicity studies of four Ciba-Geigy Corporation compounds (final report) with test data and cover letter [TSCA Submission]. (86900000262). West Lafayette, IN: Great Lakes Chemical Corporation. http://www.ntis.gov/search/product.aspx?ABBR=OTS0523254

Stapleton, HM; Eagle, S; Sjödin, A; Webster, TF. (2012). Serum PBDEs in a North Carolina toddler cohort: Associations with hand wipes, house dust and socioeconomic variables. Environ Health Perspect 120: 1049-1054. http://dx.doi.org/10.1289/ehp.1104802

U.S. EPA (U.S. Environmental Protection Agency). (1985). Toxic Substances Control Act test guidelines: Final rules [EPA Report]. Washington D.C.

U.S. EPA (U.S. Environmental Protection Agency). (1988). Recommendations for and documentation of biological values for use in risk assessment. (EPA/600/6-87/008). Cincinnati, OH: U.S. Environmental Protection Agency, National Center for Environmental Assessment. http://cfpub.epa.gov/ncea/cfm/recordisplay.cfm?deid=34855

U.S. EPA (U.S. Environmental Protection Agency). (1991). Guidelines for developmental toxicity risk assessment. (EPA/600/FR-91/001). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/guidelines-dev-toxicity-risk-assessment.htm

U.S. EPA (U.S. Environmental Protection Agency). (1996). Guidelines for reproductive toxicity risk assessment [EPA Report]. (EPA/630/R-96/009). Washington D.C.: Risk Assessment Forum, U.S. Environmental Protection Agency.

U.S. EPA (U.S. Environmental Protection Agency). (1998a). Guidelines for neurotoxicity risk assessment. (EPA/630/R-95/001F). Washington, DC: U.S. Environmental Protection Agency, Risk Assessment Forum. http://www.epa.gov/raf/publications/pdfs/NEUROTOX.PDF

U.S. EPA (U.S. Environmental Protection Agency). (1998b). Health effects test guidelines OPPTS 870.7800 immunotoxicity. (EPA 712-C-98-351). Washington, DC: Prevention, Pesticides and Toxic Substances, U.S. Environmental Protection Agency. http://www.epa.gov/ocspp/pubs/frs/publications/Test_Guidelines/series870.htm

van der Ven, LT; Verhoef, A; van de Kuil, T; Slob, W; Leonards, PE; Visser, TJ; Hamers, T; Herlin, M; Håkansson, H; Olausson, H; Piersma, AH; Vos, JG. (2006). A 28-day oral dose toxicity study enhanced to detect endocrine effects of hexabromocyclododecane in Wistar rats. Toxicol Sci 94: 281-292. http://dx.doi.org/10.1093/toxsci/kfl113

van der Ven, LTM; van de Kuil, T; Leonards, PEG; Slob, W; Lilienthal, H; Litens, S; Herlin, M; Håkansson, H; Cantón, RF; van den Berg, M; Visser, TJ; van Loveren, H; Vos, JG; Piersma, AH. (2009). Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats. Toxicol Lett 185: 51-62. http://dx.doi.org/10.1016/j.toxlet.2008.12.003

Watanabe, W; Shimizu, T; Sawamura, R; Hino, A; Konno, K; Hirose, A; Kurokawa, M. (2010). Effects of tetrabromobisphenol A, a brominated flame retardant, on the immune response to respiratory syncytial virus infection in mice. Int Immunopharmacol 10: 393-397. http://dx.doi.org/10.1016/j.intimp.2009.12.014

Weeke, J; Gundersen, HJ. (1978). Circadian and 30 minutes variations in serum TSH and thyroid hormones in normal subjects. Acta Endocrinol 89: 659-672.

Weiss, J; Wallin, E; Axmon, A; Jönsson, BA; Akesson, H; Janák, K; Hagmar, L; Bergman, A. (2006). Hydroxy-PCBs, PBDEs, and HBCDDs in serum from an elderly population of Swedish fishermen's wives and associations with bone density. Environ Sci Technol 40: 6282-6289. http://dx.doi.org/10.1021/es0610941

WHO (World Health Organization). (2012). Guidance for Immunotoxicity risk assessment for chemicals. http://www.who.int/entity/ipcs/methods/.../guidance_immunotoxicity.pdf

WIL Research Labs. (1997). A 28-day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 3/18/1997 [TSCA Submission]. (EPA/OTS Doc #86970000747). Arlington, VA: Chemical Manufacturers Association. http://www.ntis.gov/search/product.aspx?ABBR=OTS0558957

WIL Research Labs. (1998). Addendum to final report (thyroid histopathology), a 28-day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 6/18/1998 [TSCA Submission]. (86980000155). Arlington, VA: Chemical Manufacturers Association. http://www.ntis.gov/search/product.aspx?ABBR=OTS0559493

WIL Research Labs. (2001). 90-Day oral (gavage) toxicity study of HBCD in rats. (WIL-186012). Ashland, OH: WIL Research Laboratories, Inc.

WIL Research Labs. (2002). Hexabromocyclododecane (HBCD): A 90-day oral (gavage) toxicity study of HBCD in rats - final report, with cover letter dated 010302. (FYI-OTS-0102-001424). Arlington, VA: Chemical Manufacturers Association.

Wu, M; Zuo, Z; Li, B; Huang, L; Chen, M; Wang, C. (2013). Effects of low-level hexabromocyclododecane (HBCD) exposure on cardiac development in zebrafish embryos. Ecotoxicology 22: 1200-1207. http://dx.doi.org/10.1007/s10646-013-1107-4

Wu, N; Herrmann, T; Paepke, O; Tickner, J; Hale, R; Harvey, E; La Guardia, M; Mcclean, MD; Webster, TF. (2007). Human Exposure to PBDEs: Associations of PBDE Body Burdens with Food Consumption and House Dust Concentrations. Environ Sci Technol 41: 1584-1589. http://dx.doi.org/10.1021/es0620282

Yamada-Okabe, T; Sakai, H; Kashima, Y; Yamada-Okabe, H. (2005). Modulation at a cellular level of the thyroid hormone receptor-mediated gene expression by 1,2,5,6,9,10-hexabromocyclododecane (HBCD), 4,4'-diiodobiphenyl (DIB), and nitrofen (NIP). Toxicol Lett 155: 127-133. http://dx.doi.org/10.1016/j.toxlet.2004.09.005

Zeiger, E; Anderson, B; Haworth, S; Lawlor, T; Mortelmans, K; Speck, W. (1987). Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals. Environ Mutagen 9: 1-60. http://dx.doi.org/10.1002/em.2860090602

Zhang, H, ui; Pan, L; Tao, Y; Tian, S; Hu, Y. (2013). Identification and expression of differentially expressed genes in clam Venerupis philippinarum in response to environmental pollutant hexabromocyclododecane (HBCD). Exp Mar Bio Ecol 445: 166-173. http://dx.doi.org/10.1016/j.jembe.2013.03.002

Zhang, X; Yang, F; Xu, C; Liu, W; Wen, S; Xu, Y. (2008a). Cytotoxicity evaluation of three pairs of hexabromocyclododecane (HBCD) enantiomers on Hep G2 cell. Toxicol In Vitro 22: 1520-1527. http://dx.doi.org/10.1016/j.tiv.2008.05.006

Zhang, X; Yang, F; Zhang, X; Xu, Y; Liao, T; Song, S; Wang, J. (2008b). Induction of hepatic enzymes and oxidative stress in Chinese rare minnow (Gobiocypris rarus) exposed to waterborne hexabromocyclododecane (HBCDD). Aquat Toxicol 86: 4-11. http://dx.doi.org/10.1016/j.aquatox.2007.07.002

APPENDIX A. PRELIMINARY EVIDENCE TABLES AND EXPOSURE-RESPONSE ARRAYS

A.1. Data Extraction: Preparation of Preliminary Evidence Tables and Exposure-Response Arrays for Primary Studies

Key study design information, including study characteristics that inform the quality of the studies, and results from primary sources of health effects data considered pertinent for evaluating the health effects from chronic exposure to HBCD are summarized in preliminary evidence tables (Appendix A). The information in the preliminary evidence tables is also displayed graphically in preliminary exposure-response arrays. In these arrays, a significant effect (indicated by a filled circle) is based on statistical significance.

Key study design information and results from human studies are summarized in a single preliminary evidence table (Table A-1) rather than in multiple tables by health effect because the outcomes examined in these studies, including endocrine (thyroid and reproductive hormone), neuropsychological, neurobehavioral, and developmental outcomes may be inter-related. Considering the human studies as a group may provide a more integrated evaluation of the potential health effects of HBCD. In addition, human evidence will be considered together with the available animal evidence in the overall evaluation and synthesis of evidence for each health effect.

The complete list of references considered in preparation of these materials can be found on the HERO website at http://hero.epa.gov/HBCD.

A.2. Effects in Humans

Table A-1. Evidence pertaining to effects in humans

Reference and Study Design	Results
Studies in infants and children	
Eggesbø et al. (2011) (Norway, 2003-2006) Birth cohort, recruited within 2 weeks of delivery study (able and willing to provide breast milk sample), 396 randomly selected for analysis; 234 of these were after Feb 2004 when link to thyroid data became available; 193 with HBCD data (46% girls) **Exposure measures:** breast milk, collected at a median of 33 days after delivery (samples pooled over 8 consecutive mornings) HBCD detected in 67.9% of samples LOQ = 0.2 ng/g lipid Median 0.54 (range: 0.1–31) ng/g lipid **Effect measures:** TSH (whole blood spots) measured in infants 3 days after delivery; immunoassay (clinical lab) **Analysis:** Linear regression with ln TSH as a continuous outcome and logistic regression with dichotomized ln TSH (at 80thpercentile); see results column for consideration of covariates. Referent category includes all samples < LOQ; remaining 32% of population divided into 4 other categories.	Association between HBCD level in breast milk with neonatal TSH levels: 0.1 ng/g lipid: (Referent); (Referent) 0.13–0.52 ng/g lipid: -0.01 (-0.21, 0.20); 1.3 (0.3, 4.5) 0.53–0.79 ng/g lipid: 0.02 (-0.18, 0.22); 1.4 (0.3, 6.1) 0.80–1.24 ng/g lipid: 0.12 (-0.08, 0.33); 1.6 (0.4, 6.1) 1.29–31.2 ng/g lipid: 0.03 (-0.17, 0.23); 1.3 (0.3, 5.8) Per IQR increase: -0.00 (-0.02, 0.02); 1.0 (0.8, 1.1) (Adjusted Beta (95% CI)[a]; Adjusted odds ratio (95% CI)[b]) [a] outcome = ln TSH; 0.0 = no effect [b] outcome = TSH ≥80th percentile; 1.0 = no effect Adjusted for age at TSH screening, maternal BMI, county, ppDDE, HCB, delivery type, pregnancy preeclampsia and hypertension. Also evaluated but eliminated maternal education, age at delivery, Norwegian nationality, season, parity, smoking, sex, gestational age, beta-HCH, oxychlordane, and sum of all PCB congeners.
Meijer et al. (2012) (the Netherlands, COMPARE cohort, 2001-2002) Pregnancy cohort, 90 singleton, term births, 55 healthy boys, assessed at 3 months (n=55) and 18 months (n=52); 44 with HBCD measures, 45 with hormone measures, 34 with both measures **Exposure measures:** prenatal exposure, maternal serum at 35th week of pregnancy 1,2,5,6,9,10-HBCD (HBCDD) detected in 43 of 44 samples LOD 0.8 pg/g serum; LOQ = 9 pg/g serum Median 76 (range 36-180) pg/g serum or 0.7 (range: n.d.–7.4) ng/g lipid **Effect measures:** Hormones (serum, collected at 3 months) (immunoassay details in Laven et al., 2004)	Spearman correlation between HBCDD in maternal serum and free testosterone: r = -0.31 (0.05 < p-value < 0.10) Correlations with other hormones noted as not statistically significant but quantitative results were not reported No significant correlations between prenatal exposure to HBCD and testes volume or penile length were found (data not shown).

Table A-1. Evidence pertaining to effects in humans

Reference and Study Design	Results
• testosterone • sex hormone binding globulin (SHBG) • follicle stimulating hormone (FSH) • luteinizing hormone (LH) • estradiol (E2) • inhibin B Testes volume, measured by ultrasound (ages 3 and 18 months); penile length (ages 3 and 18 months) **Analysis:** Spearman correlation	
Roze et al. (2009) (the Netherlands, COMPARE cohort, 2001–2002 at baseline) Pregnancy cohort, 90 singleton, term births, 62 of 69 (90%) mother-child pairs randomly selected from the cohort for HBCDD measures in serum; children ages 5–6 years at follow-up **Exposure measures:** Prenatal exposure, maternal serum at 35th week of pregnancy 1,2,5,6,9,10-HBCD (HBCDD) detected in all samples LOD 0.8 pg/g serum; LOQ = 9 pg/g serum Median 0.8 (range: 0.3–7.5) ng/g lipids **Effect measures:** Neuropsychological tests (references for procedure provided) • Movement ABC test battery for motor performance (coordination, fine motor skills) • Developmental Coordination Disorder Questionnaire for behavior • Wechsler Preschool and Primary Scale of Intelligence, Revised for intelligence (total, verbal, performance) • Neuropsychological Assessment (NEPSY-II) for visual perception, visuomotor integration, inhibitory control • Rey's Auditory Verbal Learning test (verbal memory) • Test of Everyday Attention for Children (attention) Behavioral tests (references for procedure provided) • Child Behavior Checklist and Teacher's Report Form • Attention Deficit/Hyperactivity Disorder	*Neuropsychological measure* — *Correlation coefficient*[a] Coordination — 0.29 ($p<0.05$) Total intelligence — 0.393 ($p<0.05$) Verbal intelligence — 0.479 ($p<0.01$) [a] positive correlations indicate better outcomes. Correlations between lipid-adjusted HBCDD and outcome measure adjusted for SES, Home Observation for Measurement of the Environment HOME score, and sex. Results for correlations between other outcomes (neuropsychological, behavioral and thyroid hormone levels) were not shown, but were stated to be not statistically significant ($p>0.10$).

Table A-1. Evidence pertaining to effects in humans

Reference and Study Design	Results
questionnaire Hormones (cord blood samples, n=51, selected based on amount of sample available): T4, freeT4, rT3, T3, TSH, TBG (assay not described) **Analysis:** Pearson correlation (for normally distributed variables) or Spearman's rank correlation (for non-normally distributed variables)	
Studies in adolescents	
Kiciński et al. (2012) (Belgium, 2008-2011) Cross-sectional study, 515 adolescents (13–17 yr old) from two industrial sites and randomly selected from the general population; participation rates 22–34% in the 3 groups, sample size varies by test **Exposure measures:** Serum samples, HBCD > 75% were < LOQ (LOQ = 30 ng/L); Median <30 (range: <LOQ – 234) ng/L **Effect measures:** Neurobehavior (Neurobehavioral Evaluation System, NES-3) – computerized battery (references for procedure provided) • Continuous Performance test (attention) • Digit-Symbol test (visual scanning and information processing) • Digit Span test (working memory) • Finger Tapping (motor function) Hormones: Free T3, free T4, TSH (immunoassay not described) **Analysis:** Regression models (linear or negative binomial depending on outcome)	Beta (95% CI)[a] Continuous Performance reaction time (msec) (n=489): -3.53 (-18.72, 11.67) Continuous Performance errors of omission (%) (n=489): 27.8 (-17.5, 97.9) Continuous Performance errors of commission (%) (n=489): 21.8 (-2.5, 52.2) Digit Symbol total latency (sec) (n=340): -0.44 (-6.59, 5.72) Digit Span, Forward (n=511): 0.13 (-0.22, 0.49) Digit Span, Backward (n=499): -0.04 (-0.39, 0.31) [a]0.0 = no effect; Beta is for HBCD > LOQ versus <LOQ Linear regression models for all outcomes except Continuous Performance errors of omission and commission, where negative binomial models were used. All models adjusted for age, gender, type of education, blood lipids, smoking, parental smoking, parental education, and parental home ownership. Additional covariates evaluated included BMI, physical activity, computer use, alcohol and fish consumption, blood lead and blood PCBs, and were included based on a stepwise regression procedure. Hormone results (estimated from Figure 4 of Kiciński et al. (2012): Beta (95% CI)[b] FT3 (pg/mL): 0.08 (-0.08, 2.3) FT4 (mg/dL): -0.02 (-0.03, 0.09) TSH (%): 0.0 (-4, 13) [b]0.0 = no effect; Beta is for HBCD > LOQ versus <LOQ Linear regression models for FT3 and FT4; negative binomial model for TSH. All models adjusted for age, gender, blood lipids, BMI. Additional covariates evaluated included smoking, parental smoking, parental education, and parental

Table A-1. Evidence pertaining to effects in humans

Reference and Study Design	Results
	home ownership, physical activity, computer use, alcohol and fish consumption, blood lead and blood PCBs, and were included based on a stepwise regression procedure.
Studies in adult men	
Johnson et al. (2013) (USA, 2002–2003) Cross-sectional study, 38 men (18-54 yr old), from couples seeking infertility treatment; approximately 65% participation into general study; participation rate in the vacuum bag collection phase not reported **Exposure measures:** HBCD exposure from vacuum bag dust; three main stereoisomers of HBCD presented together. HBCD detected in 97% of samples; LOD not reported; median 246 (90th percentile 1103) ng/g dust **Effect measures:** Non-fasting blood sample (immunoassay details in Meeker et al., 2008) • testosterone (T) • sex hormone binding globulin (SHBG) • follicle stimulating hormone (FSH) • luteinizing hormone (LH) • estradiol (E2) • inhibin B • prolactin • free T4 • free T3 • thyrotropin (TSH) **Analysis:** All variables analyzed as continuous variables; Spearman's correlation between HBCD in house dust and serum hormone levels; multivariable models adjusted for age and BMI	*Correlation coefficient*[a] Free androgen index (T/SHBG) 0.46 (p=0.004) SHBG -0.35 [a] (p=0.03) [a] negative values indicate inverse correlations Results for other hormones not shown. Results from regression models not shown, but reported to be similar to bivariate correlations. Note that HBCD was not strongly correlated with other flame retardants measured (Spearman correlation coefficients ranging from -0.20 to 0.27, all p-values > 0.10)

1

A.3. Effects in Animals

The evidence tables present data from studies related to a specific outcome or endpoint of toxicity. Information in the preliminary evidence tables is also displayed graphically in preliminary exposure-response arrays. In these arrays, a significant effect is based on statistical significance, with significantly different effects at individual doses based on a pairwise comparison indicated by a filled circle, or significant dose-related trends indicated by filled circles at all doses.

A.3.1. Thyroid Effects Evidence Table and Exposure-response Array

Table A-2. Evidence pertaining to thyroid effects in animals following oral exposure to HBCD

Reference and Study Design	Results							
Thyroid hormones								
(WIL Research Labs (2002), 2001) Crl:CD(SD)IGS BR rats, 20–40/sex/group 0, 100, 300, 1,000 mg/kg-d Gavage 90 d (13 wks) with additional 28-d (4-wk) recovery period Method used to measure thyroid hormones was not reported.	*Percent change compared to control*[a]							
	Doses	0	100	300	1000			
	T_3 (wk 13)							
	M F	0% 0%	-9% -4%	-8% -9%	0% -4%			
	T_3 Recovery (wk 17)							
	M F	0% 0%	22% -1%	11% 6%	28% 17%			
	T_4 (wk 13)							
	M F	0% 0%	-19*% -9%	-20*% -17*%	-37*% -21*%			
	T_4 Recovery (wk 17)							
	M F	0% 0%	2% 14%	10% 14%	-14% 25%			
	TSH (wk 13)							
	M F	0% 0%	1043*% 396*%	1052*% 1448*%	1587*% 957*%			
	TSH Recovery (wk 17)							
	M F	0% 0%	-75% -3%	-57% -32%	-15% 24%			
van der Ven et al. (2006) Wistar rats, 5/sex/group (3–5/sex/group for thyroid hormones) 0, 0.3, 1, 3, 10, 30, 100, 200 mg/kg-d Gavage	*Percent change compared to control*[a]							
	Doses	0	0.3	1	3	10	30	100	200
	TT_3								
	M F	0% 0%	4% -8%	5% -3%	10% -11%	20% -12%	11% -19%	1% 1%	10% -10%

Reference and Study Design	Results							
28 d Thyroid hormones measured by radioimmunoassay	**TT_4**							
	M F[b]	0% 0%	1% 2%	1% -3%	23% -10%	8% -7%	5% -8%	-13% -13%	3% 26**%
Ema et al. (2008) Crl:CD(SD) rats, 24 F0/sex/group, F1 and F2 offspring produced, serum hormone levels were measured in F0 and F1 adults only (8/sex/group) 0, 150, 1,500, 15,000 ppm (mean daily intakes): F0 male: 0, 10.2, 101, 1,008 mg/kg-d F0 female: 0, 14.0, 141, 1,363 mg/kg-d F1 male: 0, 11.4, 115, 1,142 mg/kg-d F1 female: 0, 14.3, 138, 1,363 mg/kg-d Diet 10 wks prior to mating and through gestation, lactation, and for two generations (multi-generation reproductive toxicity study) Thyroid hormones measured by radioimmunoassay	*Percent change compared to control*[a]							
	Doses	0ppm	150ppm	1500ppm	15000ppm			
	T_3 F0 Adults							
	M F	0% 0%	-4% 6%	-15% 10%	-12% 2%			
	T_3 F1 Adults							
	M F	0% 0%	1% -2%	2% -10%	0% -11%			
	T_4 F0 Adults							
	M F	0% 0%	-2% 11%	-27% 6%	-38*% -31*%			
	T_4 F1 Adults							
	M F	0% 0%	-3% -1%	-6% -6%	-10% -28%			
	TSH F0 Adults							
	M F	0% 0%	0% 39*%	19% 44*%	44% 102*%			
	TSH F1 Adults							
	M F	0% 0%	-4% 48%	32% 75%	30% 67*%			
Saegusa et al. (2009) Crj:CD(SD)IGS rats, 10 dams/group, litters culled to 4/sex/dam on PND 2, F1 animals maintained for 11 wks, only male offspring evaluated for thyroid hormones 0, 100, 1,000, 10,000 ppm (TWA[c]: 0, 14.8, 146.3, 1,505 mg/kg-d) Diet GD 10–PND 20 Thyroid hormones measured by electrochemiluminescence immunoassay	*Percent change compared to control*[a]							
	Doses	0	14.8	146.3	1505			
	T_3 F1 weanling (PND 20)							
	M	0%	4%	-3%	-15*%			
	T_3 F1 adults (wk 11)							
	M	0%	-3%	-8*%	-7*%			
	T_4 F1 weanling (PND 20)							
	M	0%	-4%	9%	-4%			
	T_4 F1 adults (wk 11)							
	M	0%	2%	9%	9%			
	TSH F1 weanling (PND 20)							
	M	0%	23%	12%	30*%			
	TSH F1 adults (wk 11)							

Reference and Study Design	Results				
	M	0%	23%	13%	5%
Thyroid weight					
(WIL Research Labs (2002), 2001)) Crl:CD(SD)IGS BR rats, 10/sex/group 0, 100, 300, 1,000 mg/kg-d Gavage 90 d (13 wks) with additional 28-d (4-wk) recovery period	*Percent change compared to control*[a]				
	Doses	0	100	300	1000
	Absolute thyroid weight (wk 13)				
	M F	0% 0%	20% 14%	12% 6%	0% 15%
	Thyroid/body weight (wk 13)				
	M F	0% 0%	0% 17%	0% 0%	0% 17%
	Absolute thyroid weight (wk 17)				
	M F	0% 0%	7% 25%	-2% 36*%	-3% 37*%
	Thyroid/body weight (wk 17)				
	M F	0% 0%	20% 17%	0% 33*%	20% 33*%
Ema et al. (2008) Crl:CD(SD) rats, 24 F0/sex/group, F1 and F2 offspring produced, thyroid weight was measured in F0 and F1 adults only (13–24/sex/group) 0, 150, 1,500, 15,000 ppm (mean daily intakes): F0 male: 0, 10.2, 101, 1,008 mg/kg-d F0 female: 0, 14.0, 141, 1,363 mg/kg-d F1 male: 0, 11.4, 115, 1,142 mg/kg-d F1 female: 0, 14.3, 138, 1,363 mg/kg-d Diet 10 wks prior to mating and through gestation, lactation, and for two generations (multi-generation reproductive toxicity study)	*Percent change compared to control*[a]				
	Doses	0 ppm	150 ppm	1500 ppm	15000 ppm
	F0 adults				
	M	*significant increases in absolute and relative thyroid weight*			
	Absolute thyroid weight F1 adults				
	M F	0% 0%	0% 3%	5% 11%	19*% 24*%
	Thyroid/body weight F1 adults				
	M F	0% 0%	5% 1%	3% 9%	23*% 29*%
Saegusa et al. (2009) Crj:CD(SD)IGS rats, 10 dams/group; litters culled to 4/sex/dam on PND 2, F1 animals maintained for 11 wks (10/sex/group for thyroid weight in F0 and F1 adults) 0, 100, 1,000, 10,000 ppm (TWA[c]: 0, 14.8, 146.3, 1,505 mg/kg-d) Diet GD 10–PND 20	*Percent change compared to control*[a]				
	Doses	0	14.8	146.3	1505
	Thyroid/body weight F0 Adults				
	M F	- 0%	- 18%	- 10%	- 30*%
	Thyroid/body weight F1 Adults				
	M F	0% 0%	17% -17%	19*% -10%	28*% -6

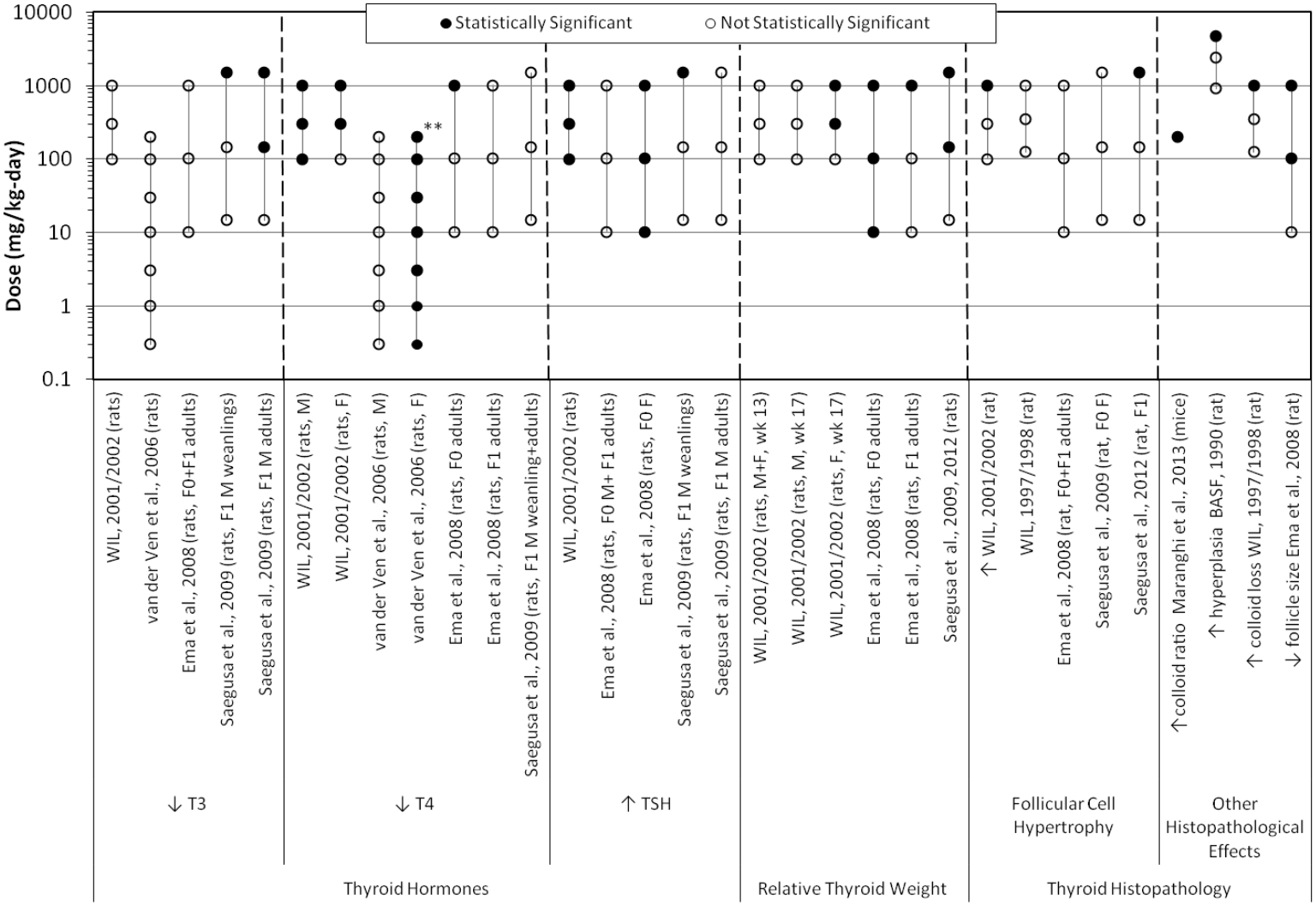
Reference and Study Design	Results				
Saegusa et al. (2012) Crj:CD(SD)IGS rats, 10 dams/group F1: 20/sex/group 0, 100, 1,000, 10,000 ppm (0, 14.8, 146.3, 1,505 mg/kg-d)[d]; 1,2,5,6,9,10-HBCD Diet GD10–PND 20	F1 Results: PND 20: statistically significant increased relative thyroid weight at 1,505 mg/kg-d (data not provided) PND 77: statistically significant increased relative thyroid weight at 146.3 and 1,505 mg/kg-d (data not provided)				
Thyroid histopathology					
(WIL Research Labs (2002), 2001)) Crl:CD(SD)IGS BR rats, 20–40/sex/group 0, 100, 300, 1,000 mg/kg-d Gavage 90 d	*Incidence*				
	Doses	0	100	300	1000
	Thyroid follicular cell hypertrophy[e]				
	M F	1/10 0/10	1/10 0/10	5/10 4/10	8/9** 7/10**
Maranghi et al. (2013) BALB/c female mice 0 (15/group), 199 mg/kg-d (10/group) Diet 28 d	Doses		0		199
	Ratio (follicle:colloid) Follicle area Colloid area		0 0% 0%		9*% -20% -26%
BASF (1990) Sprague-Dawley rats, 10/sex/group (5/sex/group for thyroid histopathology) 0, 1, 2.5, 5.0% (males: 0, 900, 2,400, 4,700 mg/kg-d; females: 0, 900, 2,300, 4,900 mg/kg-d)[c] Diet 28 d	Reported dose-related increase in thyroid hyperplasia, with highest dose characterized as having "very marked hyperplastic thyroid tissue"; adenomatous proliferation and epithelial hyperactivity in high-dose group; incidence data were not provided.				
(WIL Research Labs (1998), 1997)) Crl:CD(SD)BR rats (6–12/sex/group) 0, 125, 350, 1,000 mg/kg-d Gavage 28 d	*Incidence*				
	Doses	0	125	350	1000
	Thyroid follicular cell hypertrophy[e]				
	Minimal				
	M F	6/6 6/6	3/6 5/6	4/6 6/6	6/6 6/6
	Mild				
	M F	0/6 0/6	3/6 0/6	2/6 0/6	0/6 0/6
	Colloid loss[e]				
	Minimal				

Reference and Study Design	Results			
	M F	5/6 4/6	3/6 4/6	5/6 5/6	1/6 5/6
	Mild/Moderate				
	M F	0/6 0/6	1/6 0/6	1/6 1/6	5/6* 1/6
Ema et al. (2008) Crl:CD(SD) rats, 24 F0/sex/group, F1 and F2 offspring produced, thyroids examined in F0 and F1 adults and F1 and F2 rats at weaning 0, 150, 1,500, 15,000 ppm (mean daily intakes): F0 male: 0, 10.2, 101, 1,008 mg/kg-d F0 female: 0, 14.0, 141, 1,363 mg/kg-d F1 male: 0, 11.4, 115, 1,142 mg/kg-d F1 female: 0, 14.3, 138, 1,363 mg/kg-d Diet 10 wks prior to mating and through gestation, lactation, and for two generations (multi-generation reproductive toxicity study)	*Incidence*				
	Doses	0ppm	150ppm	1500ppm	15000ppm
	Decreased thyroid follicle size F0 adults				
	M F	0/24 0/24	0/24 0/24	6/24* 5/25*	20/23* 11/23*
	Decreased thyroid follicle size F1 adults				
	M F	0/24 0/24	0/24 1/24	2/22 5/24	11/24* 13/24*
	Thyroid follicular cell hypertrophy F0 adults				
	M F	0/24 0/24	0/24 0/24	3/24 2/24	1/24 0/24
	Thyroid follicular cell hypertrophy F1 adults				
	M F	0/24 0/24	0/24 0/24	0/24 0/24	0/24 0/24
	No treatment-related histopathological changes in thyroids in F1 or F2 weanlings.				
Saegusa et al. (2009) Crj:CD(SD)IGS rats, 10 dams/group, litters culled to 4/sex/dam on PND 2, F1 animals maintained for 11 wks (10/sex/group for thyroid histopathology) 0, 100, 1,000, 10,000 ppm (TWA[c]: 0, 15, 146, 1,505 mg/kg-d) Diet GD 10–PND 20	*Incidence*				
	Doses	0	15	146	1505
	Thyroid follicular cell hypertrophy F0 adults				
	M F	- 3/10	- 5/10	- 6/10	- 9/10
	No treatment-related histopathological changes were reported in thyroids from exposed F1 rats.				
Saegusa et al. (2012) Crj:CD(SD)IGS rats, 10 dams/group F_1: 20/sex/group 0, 100, 1,000, 10,000 ppm (0, 14.8, 146.3, 1,505 mg/kg-d)[d] Diet GD 10–PND 20	F1 Results: PND 20: increased follicular hypertrophy at 1,505 mg/kg-d (statistically significant)				

* Statistically significantly different from the control at $p < 0.05$, ** indicates p<0.01
[a] Percent change compared to control calculated as: (treated value – control value)/control value x 100.
[b] Significant dose response as reported by authors.

Reference and Study Design	Results

[c] TWA doses were estimated based on food intake and body weight data (as reported by study authors).
[d] TWA doses calculated based on food intake and body weights measured in Saegusa et al. (2009).
[e] Pairwise significance tests were conducted by EPA. For incidence data, Fisher's Exact tests were used. All statistical analyses were conducted using the freely available R statistical software (version 3.0.1). For continuous data (where means and standard deviations are provided), Student's T-tests were used.
GD = gestation day; PND = postnatal day; PNW = postnatal week; T_3 = triidothyronine; T_4 = thyroxine; TSH = thyroid stimulating hormone; TT_3 = total triiodothyronine; TT_4 = total thyroxine; TWA = time-weighted average



** Significant dose response as reported by study authors

Figure A-1. Exposure-response array of thyroid effects following oral exposure to HBCD

A.3.2. Liver Effects Evidence Table and Exposure-response Array

Table A-3. Evidence pertaining to liver effects in animals following oral exposure to HBCD

Reference and Study Design	Results				
Liver histopathology					
Kurakawa et al. (1984) SLc;B6C3F$_1$ mice, 50/sex/group 0, 100, 1,000, 10,000 ppm (0, 17, 170, 1,720 mg/kg-d)[a] Diet 18 mo	*Incidence*				
	Doses	0	17	170	1720
	Liver nodules[b]				
	M	14/50	23/50	32/50**	26/50*
	F	2/50	2/50	5/50	6/50
	Vacuolization and fatty changes[b]				
	M	8/50	9/50	31/50**	20/50*
	F	17/50	19/50	20/50	28/50*
Pharmakologisches Inst (1990b) Sprague-Dawley rats, 20/sex/group 0, 0.16, 0.32, 0.64, 1.28% (males: 0, 100, 200, 400, 900 mg/kg-d; females 0, 100, 200, 500, 950 mg/kg-d)[c] Diet 13 wks	*Incidence*				
	Doses (M) Doses (F)	0 0	100 100	200 200	400 500	900 950
	Liver fatty accumulation[b]					
	M F	4/20 10/20	8/20 11/20	11/20* 9/20	12/20* 19/20	19/20* 16/20
	Disseminated adipose droplets[b]					
	M F	1/20 5/20	0/20 4/20	1/20 7/20	2/20 6/20	6/20 10/20
(WIL Research Labs (2002), 2001)) Crl:CD(SD)IGS BR rats, 15–35/sex/group 0, 100, 300, 1,000 mg/kg-d Gavage 90 d	*Incidence*				
	Doses	0	100	300	1000
	Hepatocellular vacuolation[b]				
	M	2/10	6/10	5/10	6/9
	F	3/10	6/10	5/10	9/10*
Maranghi et al. (2013) BALB/c female mice 0 (15/group), 199 mg/kg-d (10/group) Diet 28 d	*Incidence*				
	Doses			0	199
	Vacuolation in hepatocytes			0/10	5/8**
	Pyknotic nuclei in hepatocytes			0/10	2/8
	Periportal lymphocytic infiltration			0/10	6/8**
	Tissue congestion			0/10	6/8**

Reference and Study Design	Results				
Saegusa et al. (2009) Crj:CD(SD)IGS rats, 10 dams/group; litters culled to 4/sex/dam on PND 2, F1 animals maintained for 11 wks (10/sex/group for liver histopathology) 0, 100, 1,000, 10,000 ppm (TWA[c] 0, 14.8, 146.3, 1,505 mg/kg-d) Diet GD 10–PND 20	*Incidence*				
	Doses	0	14.8	146.3	1505
	Hepatocellular vacuolar degeneration F1(PND 20)				
	M F	0/10 0/10	0/10 0/10	0/10 0/10	6/10* 6/10*
Liver weight					
Pharmakologisches Inst (1990b) Sprague-Dawley rats; 20/sex/group 0, 0.16, 0.32, 0.64, 1.28% (males: 0, 100, 200, 400, 900 mg/kg-d; females 0, 100, 200, 500, 950 mg/kg-d)[c] Diet 13 wks	*Percent change compared to control*[d]				
	Doses (M) Doses (F)	0 0	100 100	200 200	400 500	900 950
	Absolute liver weight[b]					
	M F	- 0%	- 4**%	- 8**%	- 20**%	- 30**%
	Liver/body weight[b]					
	M F	0% 0%	11**% 5**%	23**% 10**%	23**% 9**%	35**% 33**%
(WIL Research Labs (2002), 2001)) Crl:CD(SD)IGS BR rats, 15–35/sex/group (10/sex/group for liver weight) 0, 100, 300, 1,000 mg/kg-d Gavage 90 d (13 wks) with additional 28-d (4-wk) recovery period	*Percent change compared to control*[d]				
	Doses	0	100	300	1000
	Absolute liver weight (wk 13)				
	M	0%	19*%	20*%	33*%
	F	0%	22*%	31*%	53*%
	Liver/body weight (wk 13)				
	M	0%	19*%	19*%	44*%
	F	0%	24*%	24*%	48*%
	Absolute liver weight (wk 17)				
	M	0%	2%	9%	-2%
	F	0%	-6%	9%	13%
	Liver/body weight (wk 17)				
	M	0%	12*%	10*%	7%
	F	0%	-3%	11%	12%

Reference and Study Design	Results				
(WIL Research Labs (1998), 1997) Crl:CD(SD)BR rats, 6–12/sex/group (6/sex/group for liver weight) 0, 125, 350, 1,000 mg/kg-d Gavage 28 d	*Percent change compared to control*[d]				
	Doses	0	125	350	1000
	Absolute liver weight				
	M	0%	6%	13%	25*%
	F	0%	18%	29*%	40*%
	Liver/body weight				
	M	0%	10%	17*%	29*%
	F	0%	16*%	22*%	38*%
BASF (1990)[e] Sprague-Dawley rats, 5/sex/group for liver weight 0, 1, 2.5, 5.0% (males: 0, 900, 2,400, 4,700 mg/kg-d; females: 0, 900, 2,300, 4,900 mg/kg-d)[c] Diet 28 d	*Percent change compared to control*[d]				
	Doses (M) Doses (F)	0 0	900 900	2400 2300	4700 4900
	Absolute liver weight				
	M F	0% 0%	39*% 40*%	50*% 52*%	52*% 72*%
	Liver/body weight				
	M F	0% 0%	27*% 33*%	59*% 62*%	105*% 108*%
Maranghi et al. (2013) BALB/c female mice 0 (15/group), 199 mg/kg-d (10/group) Diet 28 d	*Percent change compared to control*[d]				
	Doses		0		199
	Absolute liver weight		0%		22%
	Relative liver weight		0%		29*%
Ema et al. (2008) Crl:CD(SD) rats, 24 F0/sex/group, F1 and F2 offspring produced; liver weight was assessed in all generations, 13–24/sex/group 0, 150, 1,500, 15,000 ppm (mean daily intakes): F0 males: 0, 10.2, 101, 1,008 mg/kg-d F0 females: 0, 14.0, 141, 1,363 mg/kg-d F1 males: 0, 11.4, 115, 1,142 mg/kg-d F1 females: 0, 14.3, 138, 1,363 mg/kg-d) Diet 10 wks prior to mating and through gestation, lactation, and for two generations (multi-generation reproductive toxicity study)	Doses	0ppm	150ppm	1500ppm	15000ppm
	F0 males and females				
	Increased absolute and relative liver weights were observed in F0 males (≥1,500 ppm) and females (15,000 ppm) (data not provided)				
	Absolute liver weight F1 weanlings				
	M F	0% 0%	5% 6%	12*% 17*%	20*% 21*%
	Liver/body weight F1 weanlings				
	M F	0% 0%	0% 0%	10*% 10*%	30*% 33*%
	Absolute liver weight F1 adults				
	M F	0% 0%	-2% 6%	5% 6%	14*% 15*%
	Liver/body weight F1 adults				
	M	0%	2%	3%	18*%

Reference and Study Design	Results							
	F	0%	5%	5%	21*%			
	Absolute liver weight F2 weanlings							
	M F	0% 0%	4% 1%	6% 2%	0% -4%			
	Liver/body weight F2 weanlings							
	M F	0% 0%	0% 0%	7% 5%	27*% 25*%			
Saegusa et al. (2009) Crj:CD(SD)IGS rats, 10 dams/group, litters culled to 4/sex/dam on PND 2, F1 animals maintained for 11 wks (10/sex/group for liver weight) 0, 100, 1,000, 10,000 ppm (TWA[c]: 0, 14.8, 146.3, 1,505 mg/kg-d) Diet GD 10–PND 20	*Percent change compared to control*[d]							
	Doses	0	14.8	146.3	1505			
	Liver/body weight: F1 (PND 20)							
	M F	0% 0%	4% 2%	8% 6%	27*% 28*%			
	Liver/body weight: F1 (wk 11)							
	M F	0% 0%	10*% 7%	4% 3%	2% -1%			
Liver chemistry								
van der Ven et al. (2006) Wistar rats, 3–5/sex/group for liver chemistry 0, 0.3, 1, 3, 10, 30, 100, 200 mg/kg-d Gavage 28 d	*Percent change compared to control*[d]							
	Doses	0	0.3	1	3	10	30	100	200
	T_4-UGT[f]								
	M F	0% 0%	22% 9%	11% -5%	92% -23%	67% 2%	103% 32%	175% 148%	144% 77%
	Sum of apolar liver retinoids								
	M F	0% 0%	44% 1%	21% -13%	42% -12%	19% -26%	16% -21%	-5% -7%	22% -15%
van der Ven et al. (2009) Wistar rats, 10/sex/group, F1 offspring evaluated at PND 21 (2/sex/litter) and PNW 11 (5/sex/group) 0, 0.1, 0.3, 1, 3, 10, 30, 100 mg/kg-d Diet One full spermatogenic or two full estrous cycles (males: 70 d prior to mating; females: 14 d prior to mating) and continued during pregnancy and lactation for a total of 11 wks post weaning	*Percent change compared to control*[d]							
	Doses	0	0.1	0.3	1	3	10	30	100
	Sum of apolar liver retinoids[f]								
	M F	0% 0%	7% 30%	14% 27%	32% 38%	-4% 2%	-5% 0%	-4% 9%	-19% -17%

* Statistically significantly different from the control at $p < 0.05$, ** indicates p<0.01

[a] Doses were based on standard values for body weight and food consumption in B6C3F$_1$ mice in a chronic study [i.e., average male and female body weight = 0.0363 kg and food consumption = 0.00625 kg/day; U.S. EPA (1988)].

[b] Pairwise significance tests were conducted by EPA. For incidence data, Fisher's Exact tests were used. All statistical analyses

Reference and Study Design	Results

were conducted using the freely available R statistical software (version 3.0.1). For continuous data (where means and standard deviations are provided), Student's T-tests were used.

[c] TWA doses were estimated based on food intake and body weight data (as reported by study authors).

[d] Percent change compared to control calculated as: (treated value – control value)/control value x 100.

[e] Quality of only available copy of report was difficult to read; values in tables could not be verified with certainty.

[f] Significant dose response as reported by authors.

GD = gestation day; PND = postnatal day; PNW = postnatal week; T_4-UGT = hepatic T_4-UDP (uridine diphosphate) glucuronosyltransferase; TWA = time-weighted average

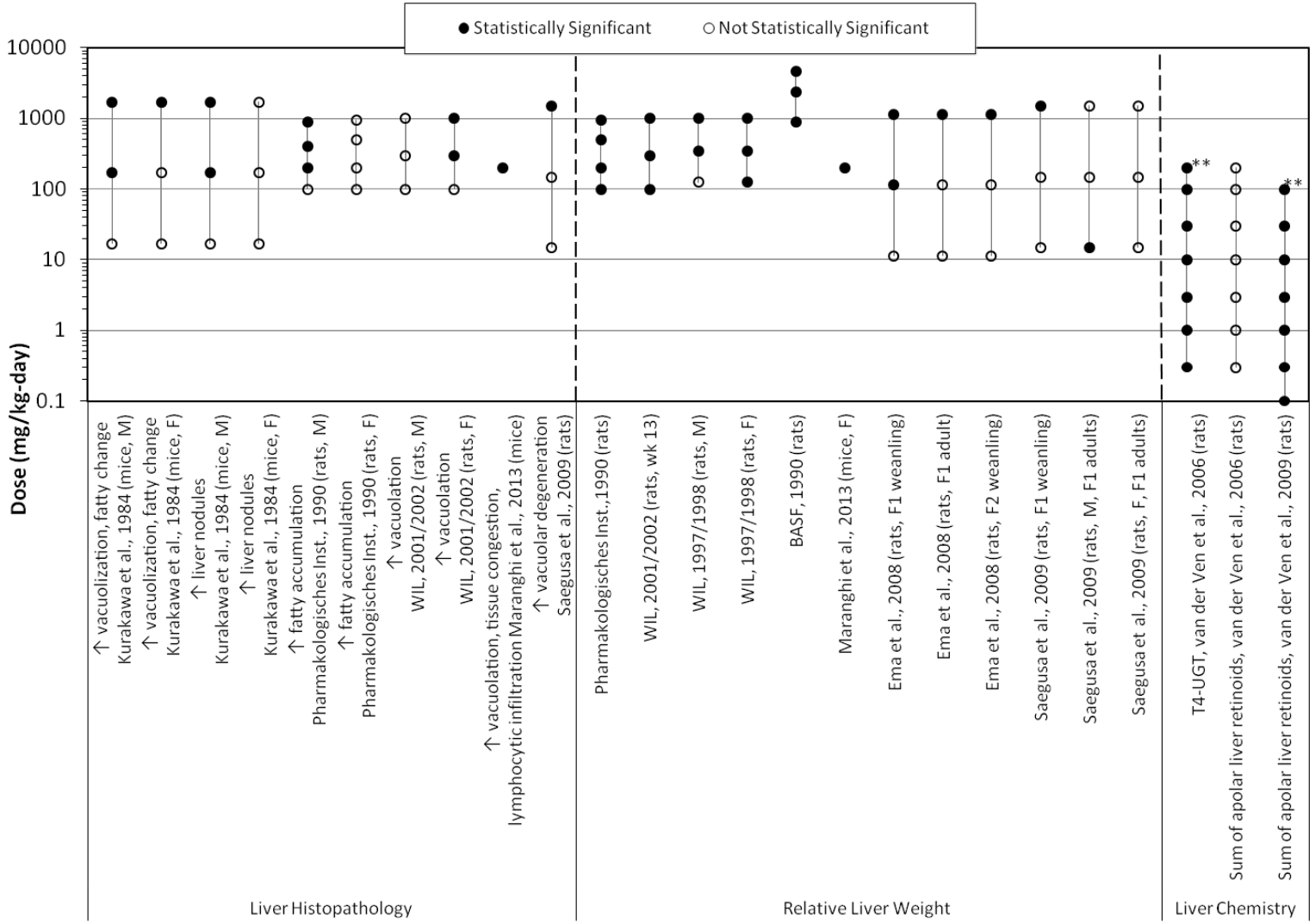


Figure A-2. Exposure-response array of liver effects following oral exposure to HBCD

A.3.3. Neurological Effects Evidence Table and Exposure-response Array

Table A-4. Evidence pertaining to neurological effects in animals following oral exposure to HBCD

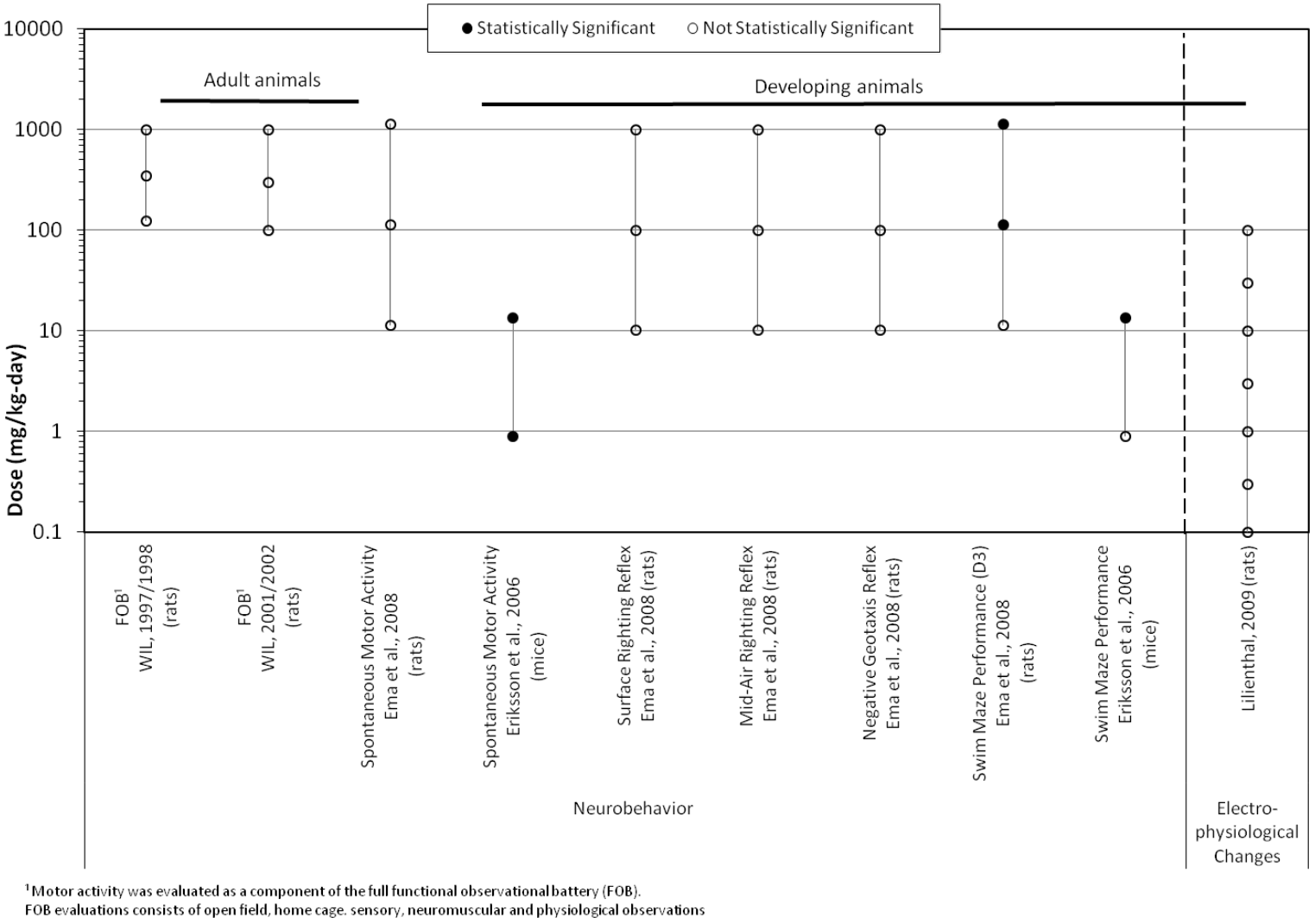
Reference and Study Design	Results[a]				
Neurobehavior					
(WIL Research Labs (2002), 2001) Crl:CD(SD)IGS BR rats, 20–40/sex/group 0, 100, 300, 1,000 mg/kg-d Gavage 90 d	No treatment-related effects were observed following FOB (home cage, handling, open field, sensory, neuromuscular, or physiological observations).				
(WIL Research Labs (1998), 1997) Crl:CD(SD)BR rats, 6–12/sex/group 0, 125, 350, 1,000 mg/kg-d Gavage 28 d	No treatment-related effects were observed following FOB (home cage, handling, open field, sensory, neuromuscular, or physiological observations).				
Ema et al. (2008) Crl:CD(SD) rats, 24 F0/sex/group; F1 and F2 offspring produced, F1 generation neurobehavior endpoints, 10/sex/group 0, 150, 1,500, 15,000 ppm (mean daily intakes): F0 male: 0, 10.2, 101, 1,008 mg/kg-d F0 female: 0, 14.0, 141, 1,363 mg/kg-d F1 male: 0, 11.4, 115, 1,142 mg/kg-d F1 female: 0, 14.3, 138, 1,363 mg/kg-d Diet 10 wks prior to mating and through gestation, lactation, and for two generations (multi-generation reproductive toxicity study)	*Percent change compared to control*[a]				
	Doses	0ppm	150ppm	1500ppm	15000ppm
	Surface righting reflex response time F1 pups				
	M F	0% 0%	-13% -23%	-22% -7%	-30*% -16%
	Mid-air righting reflex completion rate F1 pups				
	M F	0% 0%	0% 0%	0% 0%	0% 0%
	Surface righting reflex response time F2 pups				
	M F	0% 0%	-5% 4%	33% -9%	5% 61%
	Mid-air righting reflex completion rate F2 pups				
	M F	0% 0%	0% 0%	-6% -10%	0% -23*%
	Negative geotaxis reflex				
	M F	*There was no exposure effect in either generation*			
	Spontaneous motor activity *(F1 males and females)*				
	No significant difference between control and HBCD-treated groups at 4 wks of age.				
	T-maze swim test *(F1 males and females)*				

Reference and Study Design	Results[a]							
	Testing was performed at 6 weeks of age. A straight channel swim test on day 1 showed no difference in swim speed. Swim maze testing was performed on days 2-4. Male rats in the middle- and high-dose groups demonstrated statistically significant shorter elapsed time as compared to controls on day 3 of testing, but not on day 2 or 4; male rats in the high-dose group made fewer errors than control animals on day 3 of testing (but not on day 2 or 4). Female rats demonstrated no significant difference between controls and treated rats.							
Eriksson et al. (2006) NMRI mice, 10–17 males/group (3–4 litters/dose group) 0, 0.9, 13.5 mg/kg Single-dose gavage PND 10	*Spontaneous motor activity at 3 mo (n = 10/dose group):*							
	Doses	0 mg/kg	0.9 mg/kg	13.5 mg/kg				
	Locomotion							
	0–20 min	–	↓*	↓**				
	20–40 min	–	–	–				
	40–60 min	–	–	↑**				
	Rearing							
	0–20 min	–	↓*	↓**				
	20–40 min	–	–	–				
	40–60 min	–	–	↑**				
	Total activity							
	0–20 min	–	–	↓**				
	20–40 min	–	–	–				
	40–60 min	–	–	↑**				
	Morris water maze at 3 mo (10–12/dose group): Increased latency to find hidden platform (d 4) and increased time to find new platform location (d 5) at 13.5 kg/d							
Electrophysiological changes								
Lilienthal et al. (2009b) Wistar rats, 3–5/sex/group 0, 0.1, 0.3, 1, 3, 10, 30, 100 mg/kg-d Diet One full spermatogenic or two full estrous cycles (males: 70 d prior to mating; females: 14 d prior to mating) and continued during pregnancy and lactation for a total of 11 wks post weaning	*Percent change compared to control*[a]							
	Doses	0	0.1	0.3	1	3	10	30	100
	Latency of foreleg on catalepsy test								
	F1 M	0%	11%	-22%	-27%	-4%	4%	-27%	-49%
	F1 F	0%	-44%	-6%	7%	-19%	-53%	-59%	-56%
	BAEP thresholds following stimulation with click								
	F1 M	0%	-3%	-44%	9%	0%	0%	29%	47%
	F1 F	0%	7%	21%	18%	-7%	23%	11%	9%

* Statistically significantly different from the control at $p < 0.05$, ** indicates $p < 0.01$

[a] Percent change compared to control calculated as: (treated value – control value)/control value x 100.

FOB = functional observational battery; GD = gestation day; PND = postnatal day; PNW = postnatal week; TWA = time-weighted average



1

2 **Figure A-3. Exposure-response array of neurological effects following oral exposure to HBCD**

A.3.4. Developmental Effects Evidence Table and Exposure-response Array

Table A-5. Evidence pertaining to developmental effects in animals following oral exposure to HBCD

Reference and Study Design	Results							
Development								
van der Ven et al. (2009) Wistar rats, 10/sex/dose, 4–9 F1 litters/group evaluated for developmental effects 0, 0.1, 0.3, 1, 3, 10, 30, 100 mg/kg-d Diet One full spermatogenic or two full estrous cycles (males: 70 d prior to mating; females: 14 d prior to mating) and continued during pregnancy and lactation for a total of 11 wks post weaning	*Percent change compared to control*[a]							
	Doses	0	0.1	0.3	1	3	10	30	100
	Time to vaginal opening F1 offspring								
	F[b]	0%	0%	2%	4%	4%	0%	-2%	13%
	Anogenital distance (PND 4)								
	M[b]	0%	11%	2%	4%	9%	9%	-2%	17%
	F	0%	6%	-3%	0%	0%	6%	0%	3%
	PND 7 and 21: unaltered								
	Preputial separation								
	No exposure-related effect in male F1 pups								
Ema et al. (2008) Crl:CD(SD) rats, 24 F0/sex/group, F1 and F2 offspring produced; 18–24 litters/group 0, 150, 1,500, 15,000 ppm (mean daily intakes): F0 male: 0, 10.2, 101, 1,008 mg/kg-d F0 female: 0, 14.0, 141, 1,363 mg/kg-d F1 male: 0, 11.4, 115, 1,142 mg/kg-d F1 female: 0, 14.3, 138, 1,363 mg/kg-d Diet 10 wks prior to mating and through gestation, lactation, and for two generations (multi-generation reproductive toxicity study)	*Percent change compared to control*[a]							
	Doses	0ppm	150ppm	1500ppm	15000ppm			
	Viability index during lactation F0 parents/F1 offspring							
	D 0	99.6%	97.5%	98.8%	99.2%			
	D 4	95.6%	98.7%	98.7%	95.8%			
	D 21	93.2%	99.4%	98.1%	93.8%			
	Viability index during lactation F1 parents/F2 offspring							
	D 0	98.6%	97.7%	96.0%	97.8%			
	D 4	86.9%	87.3%	92.1%	68.4*%			
	D 21	85.0%	89.6%	71.3%	49.7*%			
	Pup weight during lactation F1 offspring							
	M (PND 0)	0%	2%	6%	0%			
	M (PND 4)	0%	5%	6%	-7%			
	M (PND 7)	0%	7%	3%	-5%			
	M (PND 14	0%	0%	0%	-7%			
	M (PND 21)	0%	2%	1%	-9*%			
	F (PND 0)	0%	5%	8*%	3%			
	F (PND 4)	0%	7%	8%	-4%			
	F (PND 7)	0%	10%	10%	-2%			
	F (PND 14	0%	6%	6%	-3%			
	F (PND 21)	0%	6%	7%	-6%			
	Pup weight during lactation F2 offspring							
	M (PND 0)	0%	-1%	4%	-3%			
	M (PND 4)	0%	2%	-1%	-12%			

Reference and Study Design	Results				
	M (PND 7)	0%	5%	-3%	-22*%
	M (PND 14	0%	8%	-1%	-23*%
	M (PND 21)	0%	6%	2%	-20*%
	F (PND 0)	0%	-3%	3%	-5%
	F (PND 4)	0%	-4%	-1%	-18*%
	F (PND 7)	0%	0%	-6%	-25*%
	F (PND 14	0%	0%	-6%	-23*%
	F (PND 21)	0%	2%	-2%	-20*%
	Anogenital distance				
	M (F0)	0%	1%	0%	-3%
	F (F0)	0%	3%	1%	-1%
	M (F1)	0%	0%	-2%	-5%
	F (F1)	0%	1%	1%	-6%
Saegusa et al. (2009) Crj:CD(SD)IGS rats, 10 dams/group, litters culled to 4/sex/dam on PND 2 0, 100, 1,000, 10,000 ppm; TWA[c]: 0, 14.8, 146.3, 1,505 mg/kg-d Diet (soy-free) GD 10–PND 20 (weaning)	*Percent change compared to control*[a]				
	Doses	0	14.8	146.3	1505
	Anogenital distance F1 (PND 1)				
	M	0%	2%	5%	3%
	F	0%	-9%	-6%	-5%
	Pup weight F1 (PND 1)				
	M	0%	2%	8%	1%
	F	0%	5%	12%	5%

* Statistically significantly different from the control at $p < 0.05$, ** indicates $p < 0.01$
[a] Percent change compared to control calculated as: (treated value – control value)/control value x 100.
[b] Significant dose response as reported by authors.
[c] TWA doses were estimated based on food intake and body weight data (as reported by study authors).
GD = gestation day; PND = postnatal day; TWA = time-weighted average

1

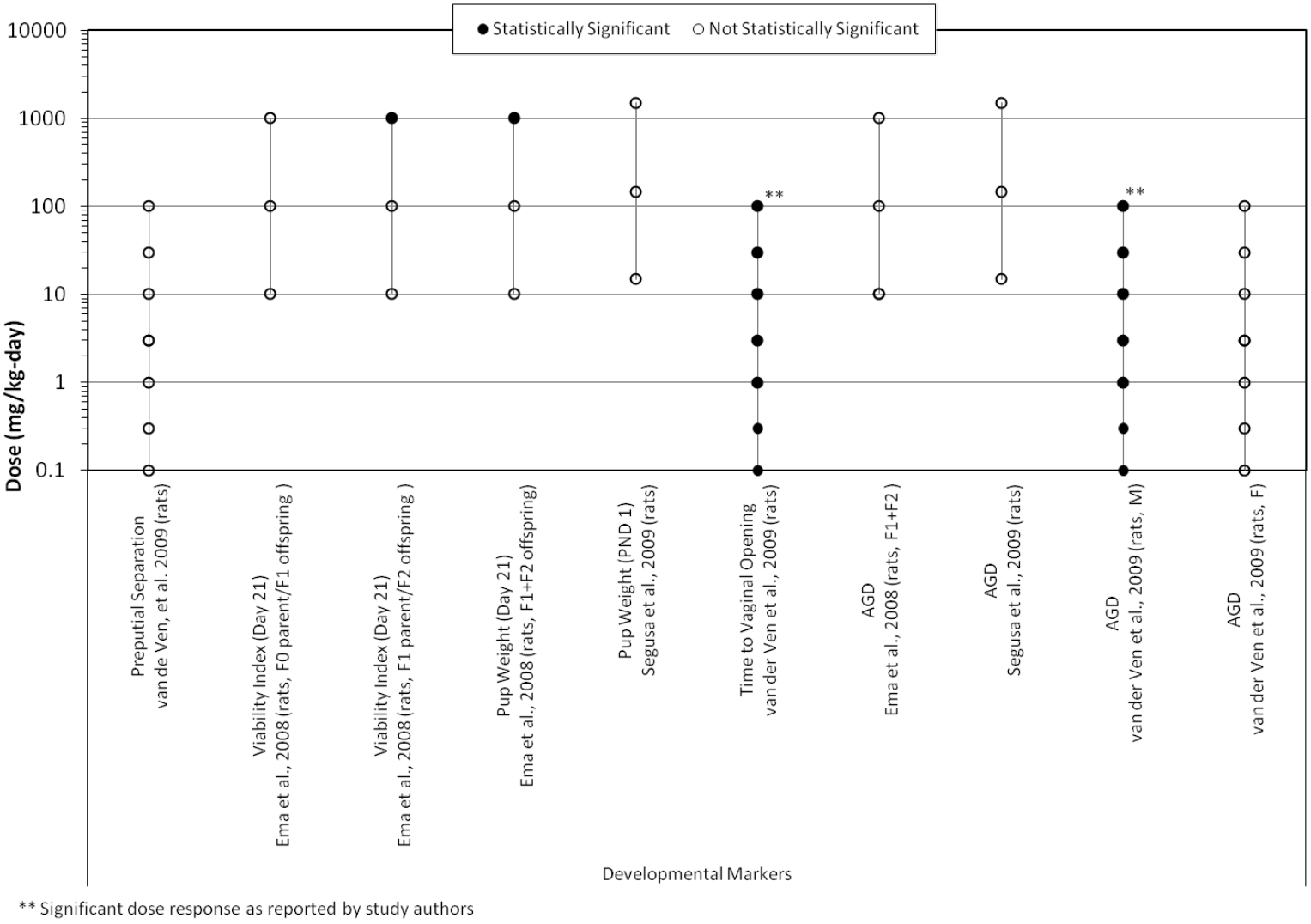


Figure A-4. Exposure-response array of developmental effects following oral exposure to HBCD

A.3.5. Reproductive Effects Evidence Table and Exposure-response Array

Table A-6. Evidence pertaining to reproductive effects in animals following oral exposure to HBCD

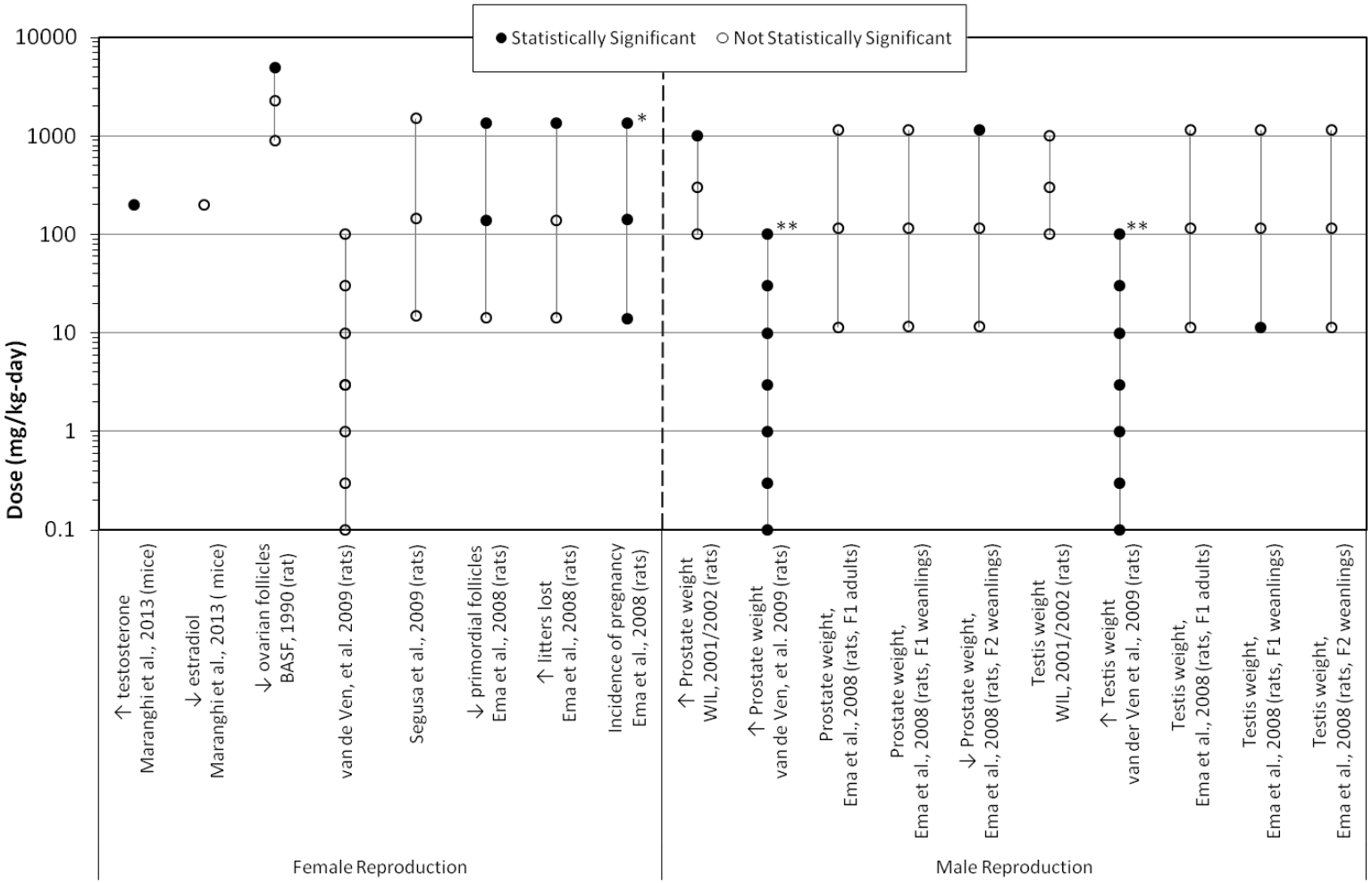
Reference and Study Design	Results		
Female reproduction			
Maranghi et al. (2013) BALB/c female mice 0 (15/group), 199 mg/kg-d (10/group) Diet 28 d	*Percent change compared to control*[a]		
	Doses	0	199
	Testosterone (T)	0%	57*%
	Estradiol (E2)	0%	-9%
	T/E2 ratio	0%	56*%
BASF (1990) Sprague-Dawley rats, 5/sex/group 0, 1, 2.5, 5.0% (males: 0, 900, 2,400, 4,700 mg/kg-d; females: 0, 900, 2,300, 4,900 mg/kg-d)[b] Diet 28 d	Decreased number of mature and developing follicles in the ovaries of high-dose group; incidence data were not provided.		
van der Ven et al. (2009) Wistar rats, 10 F0/sex/group 0, 0.1, 0.3, 1, 3, 10, 30, 100 mg/kg-d Diet One full spermatogenic or two full estrous cycles (males: 70 d prior to mating; females: 14 d prior to mating) and continued during pregnancy and lactation for a total of 11 wks post weaning	No exposure related changes in reproductive parameters, including mating success, time to gestation, gestation duration, number of implantation sites, litter size, and sex ratio.		
Saegusa et al. (2009) Crj:CD(SD)IGS rats, 10 dams/group, litters culled to 4/sex/dam on PND 2 0, 100, 1,000, 10,000 ppm (TWA[b]: 0, 14.8, 146.3, 1,505 mg/kg-d) Diet (soy-free) GD 10–PND 20 (weaning)	No exposure-related changes in reproductive parameters, including gestation length, number of implantation sites, and number of live offspring.		

Reference and Study Design	Results
Ema et al. (2008) Crl:CD(SD) rats, 24 F0/sex/group, F1 and F2 offspring produced, reproductive endpoints evaluated in F0 and F1 adults (23–24/sex/group), primordial follicles were only assessed in F1 females (10/group) 0, 150, 1,500, 15,000 ppm (mean daily intakes): F0 male: 0, 10.2, 101, 1,008 mg/kg-d F0 female: 0, 14.0, 141, 1,363 mg/kg-d F1 male: 0, 11.4, 115, 1,142 mg/kg-d F1 female: 0, 14.3, 138, 1,363 mg/kg-d Diet 10 wks prior to mating and through gestation, lactation, and for two generations (multi-generation reproductive toxicity study)	*Percent change compared to control*[a] Doses: 0 ppm; 150 ppm; 1500 ppm; 15000ppm **Primordial follicles** F1 adults F: 0%; -7%; -37*%; -36*% **Number of litters totally lost** F0 dam: no exposure-related effect reported F1 dam: 1/23; 1/23; 0/20; 8/21* **Fertility index[c] (male/female)** F0 parents/F1 offspring M: 100%*[d]; 91.7%; 90.9%; 85.7% F: 100%*[d]; 91.7%; 90.9%; 86.4% **Fertility index[c] (male/female)** F1 parents/F2 offspring M: 95.8%; 95.8%; 87.0%; 87.5% F: 95.8%; 95.8%; 87.5%; 87.5% **Incidence of pregnancy** F0 dam: 24/24*[d]; 22/24; 20/24; 19/23 F1 dam: 23/24; 23/24; 21/24; 21/24 No exposure-related changes were found in any other female reproductive parameters, including estrous cyclicity, copulation index, fertility index pre-coital interval, number of implantation sites, gestation index, delivery index, gestation length, litter size, or number and sex of live and dead pups, in F0 or F1 dams.
Male reproduction	
(WIL Research Labs (2002), 2001)) Crl:CD(SD)IGS BR rats, 20–40/sex/group 0, 100, 300, 1,000 mg/kg-d Gavage 90 d	*Percent change compared to control*[a] Doses: 0; 100; 300; 1000 **Absolute prostate weight** F1 (wk 13) 0%; 4%; 18%; 32*% **Prostate/body weight** F1 (wk 13) 0%; 3%; 17%; 43*% **Absolute testis (L+R) weight** F1 (wk 13) 0%; 3%; 2%; -2% **Testis/body weight** F1 (wk 13) 0%; 2%; 2%; 7%
van der Ven et al. (2009) Wistar rats, 10 F0/sex/group, organ weights in F1 offspring evaluated at PND 21 (2/sex/group) and wk 11 (5/sex/group), sperm parameters were	*Percent change compared to control*[a] Doses: 0; 0.1; 0.3; 1; 3; 10; 30; 100 **Absolute prostate weight**[e] F1 (wk 11) M: 0%; 11%; -14%; 11%; -14%; -12%; 2%; 36*%

Reference and Study Design	Results
evaluated at PND 21 0, 0.1, 0.3, 1, 3, 10, 30, 100 mg/kg-d Diet One full spermatogenic or two full estrous cycles (males: 70 d prior to mating; females: 14 d prior to mating) and continued during pregnancy and lactation for a total of 11 wks post weaning	**Absolute testis (L+R) weight**[e] F1 (wk 11) M: 0% -3% 2% 6% -4% -65 -1% 14*% **F1 male pups** (PND 21) *No exposure-related change in reproductive organ weights* The only exposure-related change in F1 sperm parameters was a dose-related reduction in the ratio of separate sperm heads.
Ema et al. (2008) Crl:CD(SD) rats, 24 F0/sex/group, F1 and F2 offspring produced, organ weights were assessed in all generations, 13–24/sex/group; sperm parameters were assessed in F0 and F1 adults, 23–24/group 0, 150, 1,500, 15,000 ppm (mean daily intakes): F0 male: 0, 10.2, 101, 1,008 mg/kg-d F0 female: 0, 14.0, 141, 1,363 mg/kg-d F1 male: 0, 11.4, 115, 1,142 mg/kg-d F1 female: 0, 14.3, 138, 1,363 mg/kg-d 10 wks prior to mating and through gestation, lactation, and for two generations (multi-generation reproductive toxicity study)	*Percent change compared to control*[a] Doses: 0ppm 150ppm 1500ppm 15000ppm **Absolute prostate weight** F1 adults: 0% -7% -4% -5% F1 weanlings: 0% 5% 5% -13% F2 weanlings: 0% 4% 4% -25*% **Absolute testis (L+R) weight** F1 adults: 0% -3% -3% -5% F1 weanlings: 0% 13*% 11% 1% F2 weanlings: 0% 7% 0% -19% No exposure-related changes were found in either F0 or F1 male sperm parameters.

* Statistically significantly different from the control at $p < 0.05$, ** indicates $p < 0.01$
[a] Percent change compared to control calculated as: (treated value – control value)/control value x 100.
[b] TWA doses were estimated based on food intake and body weight data (as reported by study authors).
[c] Fertility index (%) = (number of animals that impregnated a female or were pregnant/number of animals with successful copulation) x 100.
[d] Statistically significant trend test ($p < 0.05$) performed by EPA.
[e] Significant dose response as reported by authors.
GD = gestation day; PND = postnatal day; PNW = postnatal week; TWA = time-weighted average

1
2



* Statistically significant trend test ($p<0.05$) performed by the EPA
** Significant dose response as reported by study authors

Figure A-5. Exposure-response array of reproductive effects following oral exposure to HBCD

A.3.6. Immune Effects Evidence Table and Exposure-response Array

Table A-7. Evidence pertaining to immune effects in animals following oral exposure to HBCD

Reference and study design	Results							
Immune Effects								
Watanabe et al. (2010) Mouse, Balb/c, female 6-7 /group 0, 1% ppm Diet 28 days Post exposure: 5 day intranasal infection with 10^6 plaque forming units (PFU) respiratory syncytial virus	*Percent change compared to control*[a]							
	Pulmonary viral titers							
	F	0				1%		
		0%				-6%		
van der Ven et al. (2006) Rats, Wistar, male and female 5/sex/group Gavage 28 days	*Percent change compared to control*[a]							
	NK cell activity/ spleen							
	M	0	0.3	1	3	10	30	100	200
		0%	0%	-10%	3%	-13%	-47%	-14%	4%
	F	Not examined							
	Absolute $CD4^+$ cells/spleen[b]							
	M	0	0.3	1	3	10	30	100	200
		0%	7%	-7%	-21%	-21%	-36%	-21%	-29%
	F	Not examined							
	Absolute NK cells/spleen[b]							
	M	0	0.3	1	3	10	30	100	200
		0%	-21%	-25%	-4%	-15%	-44%	-40%	-46%
	F	Not examined							
	Total cells/spleen[b]							
	M	0	0.3	1	3	10	30	100	200
		0%	2%	-4%	-10%	-20%	-39%	-24%	-27%
	F	Not examined							
	Neutrophils in blood							
	M	0	0.3	1	3	10	30	100	200
		0%	-7%	44%	34%	29%	11%	67%	12%
	F	Not examined							
	Lymphocytes in blood							
	M	0	0.3	1	3	10	30	100	200
		0%	0%	-4%	-4%	-3%	0%	-5%	-1%
	F	Not examined							
	White blood cell count in blood							
	M	0	0.3	1	3	10	30	100	200
		0%	14%	23%	6%	-4%	-22%	19%	14%
	F	Not examined							

Reference and study design	Results								
	White blood cell count in bone marrow								
	M	0	0.3	1	3	10	30	100	200
		0%	0%	-40%	29%	-13%	-8%	9%	-42%
	F	Not examined							
	Relative thymus weight								
	M	0	0.3	1	3	10	30	100	200
		0%	0%	9%	0%	-18%	-18%	-9%	-9%
	F	0	0.3	1	36	10	30	100	200
		0%	-27%	0%	-13%	13%	0%	0%	0%
van der Ven et al. (2009) Rats, Wistar, male and female P generation: 10/sex/group 4 males/group 0, 0.1, 0.3, 1, 3, 10, 30, 100 mg/kg-d + standard feed control[c] Diet P generation: Males 70 days Females 14 days prior to mating and continued in dams through gestation F1 generation: exposed via milk and had access to feed of the dam Intraperitoneal injection with $2x10^9$ SRBC at 8 weeks of age; boost 15 days	*Percent change compared to control*[a]								
	SRBC antibody titers (IgM day 7)								
	M	Std	0	0.1	0.3	1	3	10	30	100
		87%	0%	20%	-93%	93%	7%	13%	33%	-7%
	F	Not examined								
	SRBC antibody titers (IgG day 21)[b]								
	M	Std	0	0.1	0.3	1	3	10	30	100
		139%	0%	100%	-6%	28%	-17%	144%	378%	161%
	F	Not examined								
	NK cell activity/ spleen								
	M	Std	0	0.1	0.3	1	3	10	30	100
		32%	0%	29%	-4%	15%	8%	5%	22%	-5%
	F	Not examined								
	Absolute $CD4^+$ cells/spleen								
	M	Std	0	0.1	0.3	1	3	10	30	100
		-6%	0%	6%	-15%	-7%	-11%	-4%	16%	-25%
	F	Not examined								
	Absolute NK cells/spleen								
	M	Std	0	0.1	0.3	1	3	10	30	100
		36%	0%	26%	0%	10%	13%	13%	33%	15%
	F	Not examined								
	Total cells/spleen								
	M	Std	0	0.1	0.3	1	3	10	30	100
		-2%	0%	10%	-8%	-4%	-10%	0%	18%	-12%
	F	Not examined								
	Neutrophils in blood[b]								
	M	Std	0	0.1	0.3	1	3	10	30	100
		13%	0%	-5%	-4%	-8%	8%	3%	12%	43%
	F	Not examined								
	Lymphocytes in blood[b]								
	M	Std	0	0.1	0.3	1	3	10	30	100
		-2%	0%	0%	0%	0%	-1%	0%	-1%	-4%
	F	Not examined								
	White blood cell count in blood[b]								
	M	Std	0	0.1	0.3	1	3	10	30	100
		-4%	0%	41%	12%	27%	-4%	16%	29%	-20%

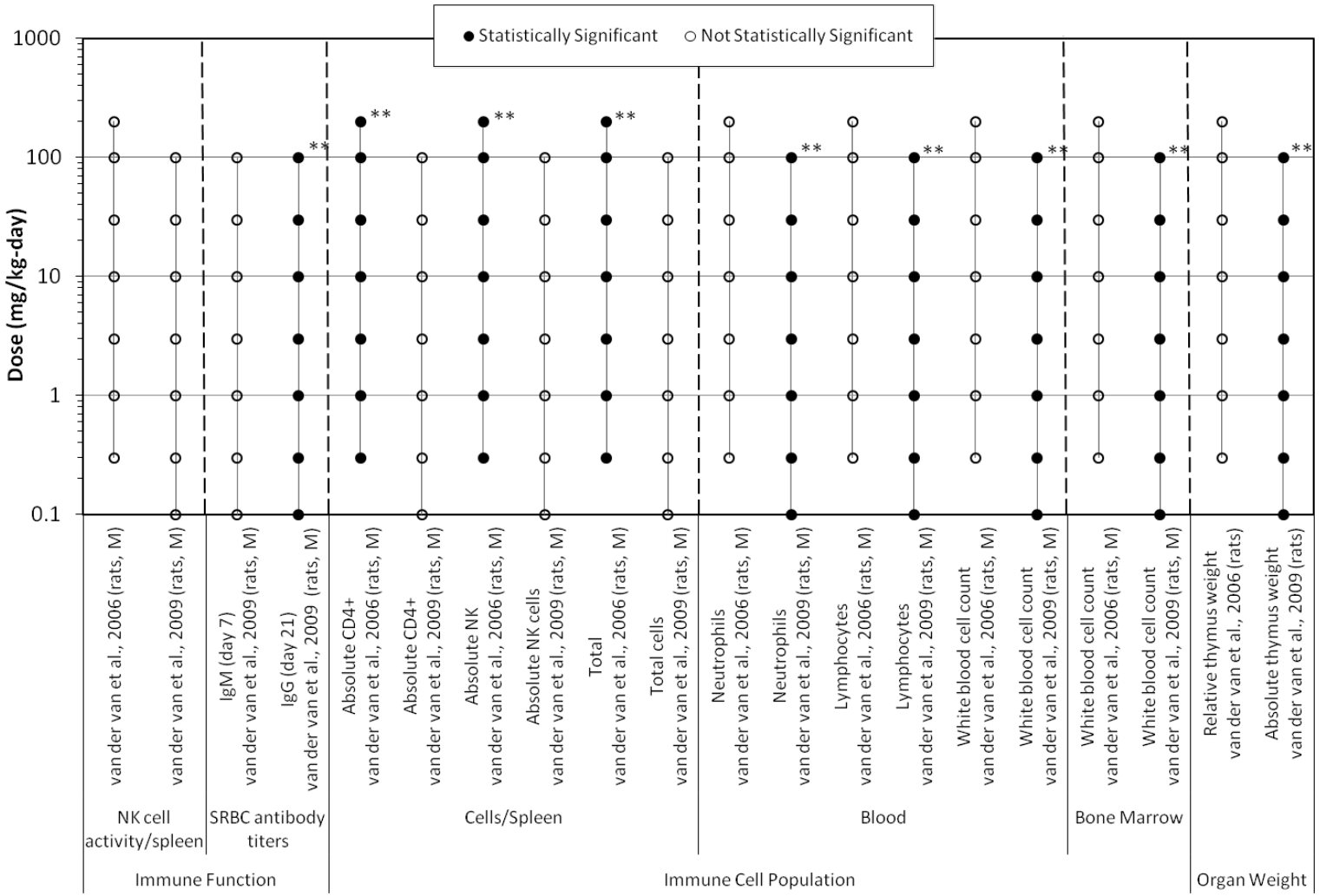
Reference and study design	Results									
	F	Not examined								
	White blood cell count in bone marrow[b]									
	M	Std	0	0.1	0.3	1	3	10	30	100
		94%*	0%	61%	83%	40%	94%	115%	72%	94%
	F	Not examined								
	Absolute thymus weight[b]									
	M	Std	0	0.1	0.3	1	3	10	30	100
		-31%**	0%	-13%	-15%	-10%	-19%	-11%	-23%	-27%
	F	Std	0	0.1	0.3	1	3	10	30	100
		-16%	0%	-16%	-18%	-14%	-2%	-8%	-10%	-24%

*Statistically significantly different from the control at $p < 0.05$, ** indicates $p < 0.01$

[a] Percent change compared to control calculated as: (treated value – control value)/control value x 100.

[b] Significant dose response as reported by authors.

[c] Significant differences between the standard feed control and test control were determined by the study authors



** Significant dose response as reported by study authors

Figure A-6. Exposure-response array of immune effects following oral exposure to HBCD

A.3.7. Information on test material used in experimental animal studies

Table A-8. Test material information

Study	Isomeric composition	Purity
BASF (1990)	Composition not reported	Not reported
Ema et al. (2008)	α: 5.8%, β: 7.9%, γ: 83.7%	99.7% purity
Eriksson et al. (2006)	Composition not reported	>98% purity
Kurakawa et al. (1984)	Composition not reported	Not reported
Lilienthal et al. (2009a)	α: 10.28%, β: 8.72%, γ: 81.02%	Not reported (noted traces of tetra- and pentabromocyclododecane)
Maranghi et al. (2013)	Composition not reported	Not reported
Pharmakologisches Inst (1990b)	Composition not reported	Not reported
Saegusa et al. (2012)	Composition not reported	>95% purity
Saegusa et al. (2009)	Composition not reported	>95% purity
van der Ven et al. (2009)	α: 10.3%, β: 8.7%, γ: 81%	Not reported
van der Ven et al. (2006)	α: 10.28%, β: 8.72%, γ: 81.01%	Not reported
Watanabe et al. (2010)	Composition not reported	Not reported
(WIL Research Labs (2002), 2001))	Composition not reported	Not reported
(WIL Research Labs (1998), 1997))	Composition not reported	Not reported

Note: Because most studies evaluated multiple endpoints and appear in multiple evidence tables, information on test materials was not added to the evidence tables to avoid unnecessary repetition.

APPENDIX B. PRELIMINARY MECHANISTIC STUDY INFORMATION

Mechanistic studies (including genotoxicity studies) identified through the literature search for HBCD (see Figure 2-1, Supporting Studies) are summarized in Table B-1. For each study, this table provides information on model system and specific assays used, route evaluated, general target tissues or systems studied, and endpoints reported. The mechanistic studies identified for HBCD consist largely of in vitro assays; entries for these studies include the cell line origin, identity, and immortalization/transformation status; culture conditions; and experimental methods.

The information presented in Table B-1 illustrates the breadth and scope of the available mechanistic data for HBCD (e.g., in vivo vs. in vitro, human vs. rodent or non-mammalian system, and level of organization – organ, system, cellular, or molecular). Mechanistic studies that did not appear to fit into one of these categories were tabulated as "other." Where possible, the following HBCD target descriptors were assigned to each study: endocrine (thyroid, development), hepatic, neurologic, reproduction and development, immunologic, and genotoxic.

This table does not include an extraction of detailed study design information (e.g., doses or concentrations, exposure durations) or assay results and, as such, does not represent an evidence table. Identifying the organ or target system will help highlight potential relationships between mechanistic information and toxicity information gathered for characterizing human health hazards related to chronic HBCD exposure.

1 **Table B-1. HBCD mechanistic studies**

Reference, effect measured, test system	Reported endpoints and assays	Target
Mammalian in vivo		
Reistad et al. (2006) *In vivo* HBCD distribution Male Wistar rats (weight from 450 to 550 g); one single intraperitoneal injection	• HBCD in rat brain after (IP) injection (brain and cerebellum; Analyses of PBDEs by GC-MS) • Brain and liver extracts–analyses of HBCD by LC-MS	Neurologic, hepatic, ADME/PBPK
BASF (2000) *In vivo* chromosomal aberrations and aneuploidy NMRI mice via i.p. injection	Micronucleus test	Genotoxicity
Mammalian in vitro		
Al-Mousa and Michelangeli (2012) *In vitro* human neurotoxicity (neuroblastoma) study SH-SY5Y human neuroblastoma cells	• Cell Viability Assay [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay; PI and staining and FACs analysis] • Caspase-3/7 activity (Ac-DEVD-AMC fluorescence) • Cytochrome c Release Assay (Immunoblotting) • Mitochondrial Membrane Potential (Rh123) • Reactive Oxygen Species (DCFH-DA) • Changes in Intracellular [Ca^{2+}] (Fluorescence) • Ca^{2+}ATPase Activity (phosphate liberation assay) • Aβ 1-42 level (β-amyloid peptide by ELISA)	Neurologic
Bastos Sales et al. (In Press) *In vitro* human neuroblastoma cell viability, global DNA methylation Human neuroblastoma (SK-N-AS cells)	• Cell viability [lactate dehydrogenase leakage (LDH); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay] • Neuroblastoma cells: global DNA methylation [(5MdC) as a percentage of the total deoxycytidines (dC + 5MdC); arbitrary primed-PCR]	Neurologic
Bastos Sales et al. (In Press) *In vitro* mouse neuroblastoma cell viability, global DNA methylation and mouse preadipocytes differentiation Mouse neuroblastoma [(Neuro-2A cells (N2A)] Mouse preadipocyte fibroblasts (3T3-L1)	All cells: Cell viability [lactate dehydrogenase leakage (LDH); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay] Mouse neuroblastoma cells: Global DNA methylation [(5MdC) as a percentage of the total deoxycytidines (dC + 5MdC); arbitrary primed-PCR] Mouse preadipocyte fibroblasts: Cell differentiation (3T3-L1 differentiation measured via flow cytometry)	Neurologic

Reference, effect measured, test system	Reported endpoints and assays	Target
Reistad et al. (2006) *In vitro* rat neurotoxicity Cultured rat cerebellar granule cells (CGC) from 7-day old pups	• Cell viability (Trypan blue exclusion) • Reactive Oxygen Species formation (DCFH-DA) • Changes in Intracellular [Ca^{2+}] (Fluorescence) • Examination of nuclear morphology (Condensed and fragmented nuclei, fluorescent probe Hoechst 33258) • Caspase-3/7 activity (Ac-DEVD-AMC fluorescence) • Internucleosomal DNA fragmentation (Apoptotic DNA ladder Kit)	Neurologic
Dingemans et al. (2009) *In vitro* neuroendocrine rat model Rat pheochromocytoma (PC12) cells	• Cell viability [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay] • Changes in Intracellular [Ca^{2+}] (Fluorescence) • Spontaneous and K^+-evoked vesicular catecholamine release (Amperometric recording)	Neurologic
Mariussen and Fonnum (2003) *In vitro* rat neurotoxicity Rat brain synaptosomes	• Synaptosomal uptake of dopamine, glutamate and GABA (Mariussen and Fonnum, 2001). • Synaptosomal accumulation of 3H-TPP+ as measure of membrane potential (Tetra[3H]phenylphosphonium Bromide)	Neurologic
An et al. (2013) *In vitro* human hepatotoxicity study Immortalized human hepatocyte L02 cell line	• Cell survival (Cell Counting kit-8) • Apoptotic cells (TUNEL assay) • Reactive oxygen species (DCFH-DA) • DNA single-strand breakages (comet assay) • Mitochondrial membrane potential (Rh123) • Changes in Intracellular [Ca^{2+}] (Fluorescence) • Protein expression (Western blot)	Hepatic
Zhang et al. (2008a) *In vitro* human hepatotoxicity study Human hepatoma cells Hep G2 (human hepatoblastoma cell line)	• Cell viability [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay] • Cell proliferation determined by total metabolic activity with resazurine [lactate dehydrogenase leakage (LDH)] • Reactive Oxygen Species (DCFH-DA)	Hepatic
Hu et al. (2009b) *In vitro* human hepatotoxicity study Human hepatoma cells Hep G2 (human hepatoblastoma cell line)	• Cell viability [lactate dehydrogenase leakage (LDH)] • Morphological observation (inverted fluorescence microscopy) • Nitric oxide synthase activity (kit) • Intra- or extracellular occurrence of nitrite (NO_2^-) (Ding method) • Reactive Oxygen Species (DCFH-DA) • Mitochondrial Membrane Potential (Rh123)	Hepatic

Reference, effect measured, test system	Reported endpoints and assays	Target
Cantón et al. (2006) *In vitro* human adrenocortical carcinoma CYP17 activity H295R human adrenocortical carcinoma cell line	• Cell viability [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay] • Combined 17α-hydroxylase and 17,20-lyase activities of CYP17 (DHEA production by radioimmunoassay)	Endocrine
Kang et al. (2012) *In vitro* human endocrine disruption Human BG-1 ovarian adenocarcinoma cell line (estrogen-dependent cell line expressing ERs, including ERα and ERβ)	• Cell viability [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay] • Gene expression (ovarian adenocarcinoma cells mRNA expression): Real-time PCR: p21, CDK2, cyclin D1 and GAPDH • Protein expression (Western blot): p21 and cyclin D1	Endocrine, Reproduction and Development
Fa et al. (2013) *In vitro* toxicity and gene expression in rat Leydig cells Primary cultures of Leydig cells obtained from 51 days old Wistar rats	• Cell viability (sulforhodamine B assay) • Change in the mitochondrial membrane potential (TMRE) • Androgen and progesterone levels in the collected incubation medium (radioimmunoassay) • cAMP and cGMP accumulation in collected media (EIA Kit) • Gene expression (Leydig cell mRNA expression): Real-time PCR (Kit): receptor B1 (Scarb-1), steroidogenic factor 1 (Sf-1), androgen receptor • (Ar) and 3-hydroxysteroid dehydrogenase 1/2 (Hsd3b1/2), cyclooxygenase 2 (Cox-2), LH receptor (Lhr), translocator protein (Tspo), steroidogenic acute regulatory protein (Star), cholesterol side chain cleavage enzyme (Cyp11a1), 17-hydroxylase/C17-20-lyase (Cyp17a1), 17-hydroxysteroid dehydrogenase 3 (Hsd17b3) and dosage-sensitive sex reversal, adrenal hypoplasia critical region, on chromosome X, gene 1 (Dax-1), Scarb-1, Star, Sf-1, Ar, Cyp11a1 and Hsd3b1/2 • Protein expression (Western blot); 30 kDa form of STAR	Endocrine, Reproduction and Development
Park et al. (2012) *In vitro* human endocrine disruption Human BG-1 ovarian adenocarcinoma cell line (estrogen-dependent cell line expressing ERs, including ERα and ERβ)	• Cell viability [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay] • Gene expression (ovarian adenocarcinoma cells mRNA expression): Real-time PCR: Cyclin D, cdk-4, p21 and GAPDH	Endocrine, Reproduction and Development

Reference, effect measured, test system	Reported endpoints and assays	Target
Yamada-Okabe et al. (2005) *In vitro* human cells that over-express thyroid receptor or estrogen receptor to monitor endocrine disruption Human HeLaTR cells that constitutively over-express human thyroid hormone receptor α1 MCF7 (human breast adenocarcinoma) cells that express human estrogen receptor α	• Cell viability (MTS) • TR and/or ER-mediated gene expression (increased luciferase activity)	Endocrine, Reproduction and Development, Thyroid
Schriks et al. (2006a) *In vitro* thyroid hormone disruption in rat Rat pituitary tumor GH3 cell line [specifically proliferates when exposed to 3,3',5-triiodo-L-thyronine (T3)]	• T-screen (Cell proliferation determined by total metabolic activity of GH3 cells with resazurine) • BrdU-cell proliferation assay (kit)	Endocrine, Thyroid
Hinkson and Whalen (2009) *In vitro* human immune defense (viral and tumor) Human NK Cells isolated from Peripheral blood from healthy adult (male and female)	• Cell viability (trypan blue exclusion) • NK cell ability to lyse tumor cells (Cr release assay) • ATP Assay (Fluorescence)	Immunologic
Hinkson and Whalen (2010) *In vitro* human immune defense (viral and tumor) Human NK Cells isolated from Peripheral blood from healthy adult (male and female)	• Cell viability (trypan blue exclusion) • Conjugation assay: target cells (NK-susceptible K562 cell (human chronic myelogenous leukemia) with bound NK cells • Cell-surface Protein Expression (FACSCalibur flow cytometer) for antibodies: anti-CD2, CD11a, CD11c, CD16, CD18, CD56, TNF-α and Fas-L, monoclonal antibody (mouseIgGκ specific for the human cell surface protein)	Immunologic
Koike et al. (2012) *In vitro* mouse immunotoxicity (splenocyte and bone marrow cytokine production and phenotype) Splenocytes and bone marrow (BM) cells prepared from atopic prone NC/Nga TndCrlJ male mice	• Cell viability (WST-1 addition) • FACS Analysis (expression of cell surface molecules via antibodies and fluorescence) • Quantitation of Cytokines in Culture Supernatants: Interferon (IFN)-g, interleukin (IL)-4, IL-17, and IL-18 (splenocyte culture supernatants); thymus- and activation-regulated chemokine, macrophage-derived chemokine and IL-12p40 levels (BMDC culture supernatants)	Immunologic
Microbiological Associates (1996) *In vitro* DNA single- and double-strand breaks Human peripheral blood lymphocytes	Chromosomal aberration test	Genotoxicity

Reference, effect measured, test system	Reported endpoints and assays	Target
Helleday et al. (1999) *In vitro* gene recombination Chinese hamster ovary (CHO) V79 Sp5 and SDP8 clones with a spontaneous partial duplication of the *hprt* gene	Intragenic recombination (reversion assay)	Genotoxicity
Ethyl Corporation (1990) *In vitro* DNA damage Primary hepatocytes from male F344 rats	Unscheduled DNA synthesis	Genotoxicity
Non-mammalian in vivo		
Aniagu et al. (2008) *In vivo* hepatotoxicity/genotoxicity model system in fish Three-spine stickleback (*Gasterosteus aculeatus*)	• Hepatic global DNA methylation	Hepatic
Zhang et al. (2008b) *In vivo* sub-lethal toxicity in fish Chinese rare minnow (*Gobiocypris rarus*) (4–6-month-old)	• Mortality • Liver: CYP1A1 (ethoxyresorufin-*O*-deethylase, EROD) and CYP2B1 (pentaoxyresorufin-*O*-dependentylase, PROD) activities (Burke and Mayer) • Brain: Reactive Oxygen Species (DCFH-DA), lipid peroxidation products (thiobarbituric acid-reactive substances, TBARS), protein oxidation *(*protein carbonyl), as well as superoxide dismutase (SOD) activity (Diagnostic Reagent Kit) and glutathione (GSH) content [(5,5-dithiobis-(2-nitrobenzoic acid) (DTNB)-oxidized GSH (glutathione disulfide, GSSG) recycling assay] • Blood: DNA damage (Comet assay) • Whole fish: Content of HBCD	Hepatic, Neurologic
Crump et al. (2010) *In vivo* exposed chick embryo liver gene expression Unincubated chicken (*G. Gallus domesticus*) eggs – exposure before hatching (prior to embryogenesis)	• Embryo viability (pipping success) • HBCD Hepatic and Cerebral Cortical Tissue (ng/g ww) concentrations • Gene expression (Hepatic mRNA expression): Real-time RT-PCR [kit: gene targets: b-actin, CYP2H1, CYP3A37, UGT1A9, L-FABP, deiodinase 2 (DI2), insulin-like growth factor-1 (IGF-1)]	Development, Hepatic

Reference, effect measured, test system	Reported endpoints and assays	Target
Deng et al. (2009) *In vivo* exposed zebrafish embryo toxicity and gene expression Wild-type (AB strain) zebrafish whole embryos	Hatching success: • Embryo malformation (pericardial edema and axial spinal curvature) • Mortality (missing heartbeat, failure to develop somites, and a non-detached tail) • Larval length For successful hatchlings: • Embryo cell apoptosis (AO staining) • Reactive Oxygen Species (DCFH-DA) • Gene expression (Whole embryo mRNA expression): Real-time PCR [Kit: gene targets: p53, Mdm2, Puma, Bax, Bcl-2, Apaf-1, caspase-3, and caspase-9] • Caspase-3 and caspase-9 activity (colorimetric assay)	Development
Du et al. (2012) *In vivo* exposed zebrafish embryo toxicity Wild-type (AB strain) zebrafish whole embryos	Hatching success: • Mortality (missing heartbeat, coagulation of the embryos, a non-detached tail and failure to develop somites) • Developmental effects (heart rate, hatching success, growth of the larvae, survival and malformation) For successful hatchlings: • Reactive Oxygen Species (DCFH-DA) • Caspase-3 and caspase-9 activity (colorimetric assay)	Development
Hu et al. (2009a) *In vivo* exposed zebrafish embryo toxicity Wild-type (AB strain) zebrafish whole embryos	• Mortality (Malformation and death) • Total protein concentration of zebrafish embryo (Bradford method) • Antioxidant Enzymes and Lipid Peroxidation [whole embryo, SOD activities and malondialdehyde (MDA) Contents, LPO (thio-barbituric assay for MDA)] • Heat shock protein (Hsp70 levels via Western Blot)	Development

Reference, effect measured, test system	Reported endpoints and assays	Target
Wu et al. (2013) *In vivo* zebrafish embryo cardiac development Wild-type (TU strain) zebrafish whole embryos	• Survival rate, whole malformation rate, and hatching rate • Morphological deformities (cardiac abnormalities, spinal deformity, altered axial curvature, and tail malformation) • Cardiac functions (arrhythmia via interbeat variability): end-diastolic volume (EDV), end-systolic volume (ESV), stroke volume (SV), and cardiac output (CO) • Apoptosis (Acridine orange (AO) staining and caspase-3 activity measurement) • Gene expression (whole zebrafish embryos): Real-time PCR: Brilliant SYBR Green QPCR reagent kit	Development
Palace et al. (2008) *In vivo* juvenile rainbow trout endocrine disruption Juvenile rainbow trout (*Oncorhynchus mykiss*) *in vivo* exposure	• Mortality and Fish growth rates • Liver somatic index (LSI) (liver weight as percentage of whole body weight) • Liver microsomal phase I [ethoxyresorufin-O-deethylase (EROD)] and II (UDPGT) biotransformation enzyme activities • Thyroid axis disruption [Free triiodothyronine (T3) and thyroxine (T4) in plasma] • Fish deiodinase activity (corresponding roughly to the D1, D2 and D3 activities in mammals); T4 outer ring deiodination • Thyroid Histopathology: Thyroid epithelial cell heights	Endocrine, Development
Palace et al. (2010) *In vivo* juvenile rainbow trout endocrine disruption Juvenile rainbow trout (*Oncorhynchus mykiss*) *in vivo* exposure	• Mortality, fish weight, length or condition • Accumulation of 1 µCi of [125I]-T4 muscle, as well as the gallbladder containing bile, thyroid gland (sampled as the entire lower jaw region), intestine (from stomach to vent), viscera (included stomach, adipose, spleen, gonad, pancreas), liver and whole blood • Deiodinase type I and II activities in individual liver microsomes	Endocrine, Development
Ronisz et al. (2004) *In vivo* juvenile rainbow trout endocrine disruption and other biomarkers Juvenile rainbow trout (*Oncorhynchus mykiss*) injected via i.p. Feral eelpout (*Zoarces viviparus*) 5 days in vivo experiment (data not shown)	• Liver microsomes and cytosol: glutathione-*S*-transferase (GST), glutathione reductase (GR) and catalase • Vitellogenin (VTG) induction in male fish plasma via ELISA (yolk-precursor produced in female fish in response to 17-estradiol, i.e., endocrine disruption biomarker) • DNA adduct formation (^{32}P-postlabelling analysis) • Liver somatic index (LSI) (liver weight as percentage of whole body weight) • Protein expression (Western blot): (PMP70 and rainbow trout only)	Endocrine, Development, Genotoxic

Reference, effect measured, test system	Reported endpoints and assays	Target
Zhang et al. (2013) *In vivo* identification and expression of differentially expressed genes (immune and detoxification defense) in clams Clam gill (Venerupis philippinarum) *in vivo* exposure (in seawater)	Gene expression (Clam gill): Real-time PCR: genes were chosen for further study with q-PCR based on novelty, relation with immunity process and detoxification process (NADH dehydrogenase subunit 1; Cytochrome c oxidase subunit 1; Purine nucleoside phosphorylase; Hemocyanin subunit 2; C-type lectin 3; Ferritin; Catalase; Elongation factor 1-alpha; Dihydrodiol dehydrogenase)	Immunologic
Non-mammalian in vitro		
Crump et al. (2008) *In vitro* chick embryo liver cells, gene expression Cultured chicken embryonic hepatocytes (CEHs) – exposure after hatching	• Cell viability [Calcein-acetoxymethylester (AM) assay] • Total RNA (TRIzol reagent Kit) • cDNA synthesis (Superscript II kit) • Gene Expression (hepatic mRNA expression): Real-time RT-PCR [kit: gene targets: b-actin, CXR, CYP2H1, CYP3A37, UGT1A9, TR-α, TTR, deiodinase (DI) 1, 2, and 3, myelin basic protein (MBP), THRSP14-a, and L-FABP]	Development
Kling and Förlin (2009) *In vitro* Zebrafish liver cell proteomic analyses Zebrafish liver (ZFL) cell test system	• Cell viability [lactate dehydrogenase leakage (LDH)] • Two-dimensional gel electrophoresis of extracted proteins from ZFL cells (63 significant responses)	Development
Schriks et al. (2006b) *In vitro* thyroid hormone disruption Xenopus laevis tadpole tail tip regression (regression induced by 3,3',5-triiodo-L-thyronine (T3) exposure) in premetamorphic tadpoles (developmental stage 52–53)	7-day exposure of tails: • Negative effects (fungal infections) • Tail regression	Endocrine, Development
Pharmakologisches Inst (1990a) *Salmonella typhimurium* TA98, TA100, TA1537	Gene mutation	Genotoxicity
Industrial Bio-Test Laboratories (1990) *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Gene mutation	Genotoxicity
Huntingdon Research Centre (1990) *S. typhimurium* TA98, TA100, TA1535	Gene mutation	Genotoxicity

Reference, effect measured, test system	Reported endpoints and assays	Target
Zeiger et al. (1987) *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Gene mutation	Genotoxicity
SRI International (1990) *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538	Gene mutation	Genotoxicity
Ogaswara et al. (1983) *S. typhimurium* TA92, TA94, TA98, TA100, TA1535, TA1537	Gene mutation	Genotoxicity
Ethyl Corporation (1990a) *S. typhimurium* TA98, TA100, TA1535, TA1537	Gene mutation	Genotoxicity
Litton Bionetics (1990) *S. typhimurium* TA98, TA100, TA1535, TA1537, TA1538 *Saccharomyces cerevisiae* D4	Gene mutation	Genotoxicity
Other		
Sakai et al. (2009) *In vitro* assay for CAR ligand activity in Baikal seal Baikal seal (*Pusa sibirica*) *in vitro* reporter gene assay (Comparison to mouse)	Ligand-dependent transcriptional activation of constitutive active/androstane receptor (CAR) Potency (CAR cDNA clones from the Baikal seal and mouse used for *in vitro* reporter gene assay)	Undetermined
Schriks et al. (2007) *In vitro* thyroid hormone dysruption in reporter gene assays (monkey cells) Transient transfection assays; Green monkey kidney fibroblast (CV-1) cells transiently transfected with *Xenopus* TRs and a luciferase reporter (TRα/β-specific reporter gene assays)	• Cell viability (assumed from prior assays) • Effects on T3 (EC50)-induced activation of TRs	Thyroid

Reference, effect measured, test system	Reported endpoints and assays	Target
Ibhazehiebo et al. (2011) *In vitro* neurotoxicity in reporter gene assays and newborn rat cultures Transient transfection-based reporter gene assays [Green monkey kidney fibroblast (CV-1) cells] Interaction of Thyroid hormone receptor with Thyroid hormone response element (TRE) Purkinje cells in primary cerebellar culture derived from newborn rat	• Cell viability (CV-1 cells, Trypan blue exclusion) • TR-mediated transcription using the transient transfection-based reporter gene assay in CV-1 cells • TR binding to TRE (liquid chemiluminescent DNA pull down assay in vitro • TH-induced dendrite arborization of Purkinje cells	Neurologic, Endocrine, Thyroid
Harju et al. (2007) Quantitative structure–activity relationships (QSARs) based on *in vitro* potencies	• Basis: *In vitro* activities (e.g., chemically activated luciferase expression reporter gene assay): Androgen, progesterone, estrogen, and dioxin (aryl hydrocarbon) receptors, plus competition with thyroxine for its plasma carrier protein (transthyretin), inhibition of estradiol sulfation via sulfotransferase, and rate of metabolization • Physicochemical parameters: Frontier molecular orbitals, molecular charges, polarities, log octanol/water partitioning coefficient, and two- and three-dimensional molecular properties • Experimental properties: Individual ultraviolet spectra (200–320 nm) and retention times on three different high-performance liquid chromatography columns and one nonpolar gas chromatography column	QSAR predictions
Fernandez Canton et al. (2005) Human adrenocortical carcinoma cell line (H295R)	Abstract only	Abstract only

1

2