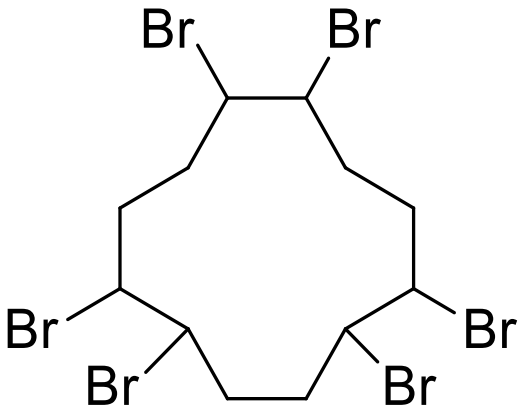
United States
Environmental Protection Agency

Office of Chemical Safety and
Pollution Prevention

Draft Risk Evaluation for Cyclic Aliphatic Bromides Cluster (HBCD)

Systematic Review Supplemental File:

Data Quality Evaluation of Human Health Hazard Studies – Animal, In Vitro and Epidemiological Studies



CASRN	NAME
25637-99-4	Hexabromocyclododecane
3194-55-6	1,2,5,6,9,10-Hexabromocyclododecane
3194-57-8	1,2,5,6-Tetrabromocyclooctane

June 2019

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1 Acute Toxicity Studies

1.1 Animal toxicity evaluation results of 1990 acute oral study for mortality, body weight outcomes

Study reference:	(1990). LETTER FROM AMERIBROM INC TO US EPA REGARDING 8D SUBMISSION FOR HEXABROMOCYCLODODECANE WITH ATTACHMENTS HERO ID: 1928284					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	The test substance was identified by abbreviation.	Medium	2	2	4
	2. Test Substance Source	The source of the test substance, including manufacturer, was not specifically reported. Lot number was not reported.	Low	3	1	3
	3. Test Substance Purity	Purity and grade were not reported and there was no analysis conducted for measurement of impurities, if present.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Use of a control group was not reported, but is not required for studies of this type and outcome	Low	3	2	6
	5. Positive Controls		Not Rated (NR)	NR	NR	NR
	6. Randomized Allocation	The study authors did not report how animals were allocated to study groups but there was only one group.	NR	NR	NR	NR
Exposure Characterization	7. Preparation and Storage of Test Substance	The study authors reported some details on test item preparation, but they were incomplete (e.g., time of stirring, temperature, etc.) and the storage conditions were not reported,	Low	3	1	3

Study reference:	(1990). LETTER FROM AMERIBROM INC TO US EPA REGARDING 8D SUBMISSION FOR HEXABROMOCYCLODODECANE WITH ATTACHMENTS HERO ID: 1928284					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	8. Consistency of Exposure Administration	A few details were reported that indicted that dosing methods were equivalent (e.g., similar dosing volumes at 10 mL/kg), but insufficient details were reported to allow determination of whether exposure administration was consistent.	Low	3	1	3
	9. Reporting of Doses/Concentrations	Administered dose level was reported.	High	1	2	2
	10. Exposure Frequency and Duration	The exposure frequency and duration were incompletely reported to allow a determination of whether they were suitable. Stated to be an acute study though, so suggests one exposure.	Low	3	1	3
	11. Number of Exposure Groups and Dose Spacing	Only one dose level was tested, but this is acceptable for studies of this type.	High	1	1	1
	12. Exposure Route and Method	The route of exposure was reported and was suited to the test substance.	High	1	1	1
Test Organism	13. Test Animal Characteristics	The test animal source, life stage, and starting body weight were not reported; species, strain, and sex were reported.	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate and/or if differences existed between the exposed and control groups. These deficiencies may have a substantial impact on the results.	Low	3	1	3

Study reference:	(1990). LETTER FROM AMERIBROM INC TO US EPA REGARDING 8D SUBMISSION FOR HEXABROMOCYCLODODECANE WITH ATTACHMENTS HERO ID: 1928284					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	The number of animals was appropriate for the study type and outcome analysis.	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Details on the outcome assessment methodology were incompletely reported (e.g., the frequency of observations during the post-exposure observation period). Due to incomplete reporting, it's not clear whether methods were sensitive for the outcomes of interest other than non-lethal outcomes	Low	3	2	6
	17. Consistency of Outcome Assessment	Consistency of the outcome assessments was not adequately reported for meaningful interpretation of results. These are serious flaws that make the study unusable.	Unacceptable	4	1	4
	18. Sampling Adequacy	Details regarding sampling of outcomes were not reported and this deficiency is likely to have a substantial impact on results.	Low	3	1	3
	19. Blinding of Assessors		Not Rated	NR	NR	NR
	20. Negative Control Response		Not Rated	NR	NR	NR
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	Lack of reporting of initial body weights and whether there were any differences among the study groups in this or other parameters is considered to have a substantial impact on the results.	Low	3	2	6

Study reference:	(1990). LETTER FROM AMERIBROM INC TO US EPA REGARDING 8D SUBMISSION FOR HEXABROMOCYCLODODECANE WITH ATTACHMENTS HERO ID: 1928284					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	22. Health Outcomes Unrelated to Exposure	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted	Low	3	1	3
Data Presentation and Analysis	23. Statistical Methods		Not Rated	NR	NR	NR
	24. Reporting of Data	Data reporting was minimal and data on outcomes of exposure were reported in the text only.	Low	3	2	6
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**26**	**65**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**2.5000**	**Overall Score (rounded):**	**2.5[1]**
		Overall Quality Level:		**Unacceptable[1]**		
Comment:	**The report provides minimal details on methodology and results; however, the results for this acute oral toxicity study may be useful in a weight of evidence with other similar studies.** **Footnote:** **[1] Consistent with our *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one of the metrics was rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.**					

1.2 Animal toxicity evaluation results of 1990 study for primary skin irritation study on irritation outcomes

Study reference:	(1990). LETTER FROM AMERIBROM INC TO US EPA REGARDING 8D SUBMISSION FOR HEXABROMOCYCLODODECANE WITH ATTACHMENTS HERO ID: 1928284					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	The test substance was identified by abbreviation. and a trade name.	Medium	2	2	4
	2. Test Substance Source	Test substance source was reported.	High	1	1	1
	3. Test Substance Purity	Purity and grade were not reported and there was no analysis conducted for measurement of impurities, if present.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Use of a control group was not reported, but is not required for studies of this type and outcome	Low	3	2	6
	5. Positive Controls		Not Rated	NR	NR	NR
	6. Randomized Allocation	The study authors did not report how animals were allocated to study groups but there was only one group.	Not Rated	NR	NR	NR
Exposure Characterization	7. Preparation and Storage of Test Substance	Test substance preparation was reported; however, storage was not reported.	Medium	2	1	2
	8. Consistency of Exposure Administration	The study reported consistent exposure administration; however, some details were lacking, such whether the exposures occurred at the same approximate time for all animals.	Medium	2	1	2
	9. Reporting of Doses/Concentrations	Administered dose level was reported.	High	1	2	2
	10. Exposure Frequency and Duration	Exposure frequency and duration were reported.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	Only one dose level was tested, but this is acceptable for studies of this type.	High	1	1	1

Study reference:	(1990). LETTER FROM AMERIBROM INC TO US EPA REGARDING 8D SUBMISSION FOR HEXABROMOCYCLODODECANE WITH ATTACHMENTS HERO ID: 1928284					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	12. Exposure Route and Method	The route of exposure was reported and was suited to the test substance.	High	1	1	1
Test Organism	13. Test Animal Characteristics	Test animal source, life stage, initial body weight, species, strain, and sex were reported; test animal was from a laboratory-maintained colony	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Husbandry conditions were reported, including lighting, temperature, and humidity.	High	1	1	1
	15. Number per Group	The number of animals per study group (six) and number of groups (one) was acceptable for the study type and outcomes of interest.	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	The outcome assessment methodology addressed or reported the intended outcomes) of interest and was sensitive for the outcomes(s) of interest.	High	1	2	2
	17. Consistency of Outcome Assessment	Details of the outcome assessment protocol were reported for some outcomes, including time points for post-exposure observations, and were the same across all groups.	Medium	2	1	2
	18. Sampling Adequacy	Details regarding sampling for the outcomes of interest were partially reported (e.g., sampling for general condition was not indicated, such as how many animals were examined.	Medium	2	1	2
	19. Blinding of Assessors		Not Rated	NR	NR	NR

Study reference:	(1990). LETTER FROM AMERIBROM INC TO US EPA REGARDING 8D SUBMISSION FOR HEXABROMOCYCLODODECANE WITH ATTACHMENTS HERO ID: 1928284					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	20. Negative Control Response		Not Rated	NR	NR	NR
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	No initial differences in body weight were reported within the treatment group and there were no other reported differences that could influence the outcome assessment	Medium	2	2	4
	22. Health Outcomes Unrelated to Exposure	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted	Low	3	1	3
Data Presentation and Analysis	23. Statistical Methods		Not Rated	NR	NR	NR
	24. Reporting of Data	There were some deficiencies in reporting of data (e.g., initial body weights were based on a range. rather than actual values.)	Low	3	2	6
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**26**	**46**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.7692**	**Overall Score: Nearest *:**	**1.8**
		Overall Quality Level:		**Medium**		

1.3 Animal toxicity evaluation results of Eriksson et al 2006 for oral neurodevelopmental study (single dose post-natal day 10) study on neurological/behavior, growth (early life) and development outcomes

Study reference:	**Eriksson, P.,Fischer, C.,Wallin, M.,Jakobsson, E.,Fredriksson, A. (2006). Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD) Environmental Toxicology and Pharmacology, 21(3), 317-322** **HERO ID: 787660**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Characterized as a mixture containing three diastereoisomers alpha-, beta-, and gamma-HBCD.	High	1	2	2
	2. Test Substance Source	Prepared from a commercial mixture, but the manufacturer and lot/batch number were not given. Analytical verification is not described.	Low	3	1	3
	3. Test Substance Purity	>98%	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Negative vehicle controls were used.	High	1	2	2
	5. Positive Controls	Positive controls were not needed for neurodevelopmental studies.	Not Rated	NR	NR	NR
	6. Randomized Allocation	Randomly selected from 3-4 different litters from each treatment group.	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Preparation was well described and appropriate. Single dose study, therefore prolonged storage is not a concern.	High	1	1	1
	8. Consistency of Exposure Administration	Details of exposure administration were reported, and exposures were administered consistently across study groups in a scientifically sound manner (dose given via a PVC tube).	High	1	1	1
	9. Reporting of Doses/Concentrations	Gavage doses were reported as both mg/kg and µmol/kg.	High	1	2	2
	10. Exposure Frequency and Duration	Administered as a single dose during a critical period (on PND 10) in neonatal development of the mouse brain.	High	1	1	1

Study reference:	**Eriksson, P.,Fischer, C.,Wallin, M.,Jakobsson, E.,Fredriksson, A. (2006). Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD) Environmental Toxicology and Pharmacology, 21(3), 317-322** **HERO ID: 787660**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Number of Exposure Groups and Dose Spacing	2 doses plus control. A justification was not provided for the doses selected, but the results suggest they were appropriate.	Medium	2	1	2
	12. Exposure Route and Method	The route and method of exposure were reported and were suited to the test substance.	High	1	1	1
Test Organism	13. Test Animal Characteristics	Species, strain and age of neonatal mice was specified.	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Most husbandry conditions were reported and were adequate and similar for all groups. Humidity was not reported. But this is unlikely to have a substantial impact on the results.	Medium	2	1	2
	15. Number per Group	The number of animals per study group was reported, appropriate for the study type and outcome analysis, and consistent with studies of the same or similar type (10/group or 12-17/group)	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Standard tests of spontaneous behavior and learning and memory.	High	1	2	2
	17. Consistency of Outcome Assessment	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups (e.g., at the same time after initial exposure) using the same protocol in all study groups.	High	1	1	1

Study reference:	**Eriksson, P.,Fischer, C.,Wallin, M.,Jakobsson, E.,Fredriksson, A. (2006). Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD) Environmental Toxicology and Pharmacology, 21(3), 317-322** **HERO ID: 787660**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	18. Sampling Adequacy	It is difficult to discern definitively but based on the methods description and a statistical paper published explaining the methods used (Eriksson 2005, The Toxicologist) it appears that the pup was used as a statistical unit. While this is less important because the mice were not exposed in utero, it still ignores known litter effects, as documented in (Holsen et al, 2008). Additionally, Holson et al 2008 recommends examining both sexes, while this study only examines males.	Low	3	1	3
	19. Blinding of Assessors	Blinding was not reported; however, outcomes were objective.	Medium	2	1	2
	20. Negative Control Response	The biological responses of the negative control group(s) were adequate.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	There were no significant deviations in body weight gain in HBCDD-treated mice compared with the vehicle-treated mice.	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group	Low	3	1	3
Data Presentation and Analysis	23. Statistical Methods	The specifics of analyzing pups as opposed to litters were not explicitly explained and failing to account for litter effects could have a large statistical impact on results.	Low	3	1	3

Study reference:	**Eriksson, P.,Fischer, C.,Wallin, M.,Jakobsson, E.,Fredriksson, A. (2006). Impaired behaviour, learning and memory, in adult mice neonatally exposed to hexabromocyclododecane (HBCDD) Environmental Toxicology and Pharmacology, 21(3), 317-322** **HERO ID: 787660**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	24. Reporting of Data	Data for exposure-related findings were presented for all outcomes by exposure group and sex.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**41**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**NR**	**Overall Score: Nearest *:**	**NR**
		Overall Quality Level:		**Medium**		
Study Quality Comment:	**The reviewer downgraded this study. They noted: Downgraded because the statistical methods are inappropriate based on proper methods for DNT studies according to other publications (e.g. Holman et al, 2008, Neurotoxicology and Teratology) Note: The original calculated score for this study was 1.4. This value is not presented above because the final rating was changed based on professional judgement.**					

1.4 Animal toxicity evaluation results of IRDC 1978 for acute toxicity studies (oral, dermal and ocular) study on gastrointestinal, irritation, and skin and connective tissues outcomes

Study reference:	**IRDC, (1978). Acute toxicity studies in rabbits and rats with residue of hexabromocyclododecane with attachments and cover letter dated 030178** **HERO ID: 787686**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	The test substance was identified as residue of HBCD (FM 100 residue). EPA requested additional information for the TSCA 8e submitter (Velsicol Chemical Corp.) as follows: "0088-Please provide information concerning the composition and physical/chemical properties of the "FM 100 Residue" which was tested. Of particular interest in this regard is the amount of hexabromocyclododecane present in the residue. Available toxicity data on hexabromocyclododecane would be useful for correlation purposes." This information is not contained in the pdf. The test substance identity and form cannot be determined from the information provided	Unacceptable	4	2	8
	2. Test Substance Source	The manufacturer was reported without batch or lot no.	Medium	2	1	2
	3. Test Substance Purity	Purity was not reported but is expected to be low because the 2 samples of the residue had different physical descriptions.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	No vehicle was used for irritation studies. Negative controls are not used for acute toxicity/lethality studies.	Not Rated	NR	2	NR
	5. Positive Controls	Positive controls are not required for irritation or acute toxicity/lethality studies.	Not Rated	NR	1	NR

Study reference:	**IRDC, (1978). Acute toxicity studies in rabbits and rats with residue of hexabromocyclododecane with attachments and cover letter dated 030178** **HERO ID: 787686**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	6. Randomized Allocation	The study did not report how animals were allocated to study groups.	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	Information on preparation and storage was not reported.	Unacceptable	4	1	4
	8. Consistency of Exposure Administration	Details of exposure administration were reported.	High	1	1	1
	9. Reporting of Doses/Concentrations	Doses were reported mg/kg in oral acute toxicity studies in rabbits. But the concentration of the test chemical dose (mg) exposed to rabbits for eye or skin irritation study was not specified. Only volume (mL) was provided.	Low	3	2	6
	10. Exposure Frequency and Duration	Adequate follow up time for examinations for all experiments.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	5 dose groups dermal acute; 6 dose groups oral acute.	High	1	1	1
	12. Exposure Route and Method	The route and method of exposure were reported and were suited to the test substance.	High	1	1	1
Test Organism	13. Test Animal Characteristics	Species, strain and starting body weight were provided (commercial source, rats and rabbits).	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Temperature and humidity controls. Compliance with animal care guidance was indicated.	Medium	2	1	2
	15. Number per Group	4-5/sex for oral acute; 2/sex/group for dermal acute; adequate numbers for irritation.	Medium	2	1	2

Study reference:	**IRDC, (1978). Acute toxicity studies in rabbits and rats with residue of hexabromocyclododecane with attachments and cover letter dated 030178** **HERO ID: 787686**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	16. Outcome Assessment Methodology	EPA requested further information from the TSCA 8e submitter (Velisicol Chemical Corp.) as follows: "Please describe any gross pathological findings or clinical observation made on the test animals."	Medium	2	2	4
	17. Consistency of Outcome Assessment	Details of the outcome assessment protocol were reported.	High	1	1	1
	18. Sampling Adequacy	Details regarding sampling for the outcome(s) of interest were reported and the study used adequate sampling for the outcome(s) of interest.	High	1	1	1
	19. Blinding of Assessors	Information in the study report did not report whether assessors were blinded to treatment group for objective outcomes	Low	3	1	3
	20. Negative Control Response	No negative controls	Not Rated	NR	NR	NR
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	There were no reported differences among the study groups in initial body weight that could influence the outcome assessment. , Information on food or water intake, or respiratory rate was not reported.	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.	Low	3	1	3
Data Presentation and Analysis	23. Statistical Methods	Provided references for statistical methods.	High	1	1	1

Study reference:	**IRDC, (1978). Acute toxicity studies in rabbits and rats with residue of hexabromocyclododecane with attachments and cover letter dated 030178** **HERO ID: 787686**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	24. Reporting of Data	Data for exposure-related findings were presented for all outcomes by exposure group and sex.	High	1	2	2
High: >=1 and <1.7 **Medium: >=1.7 and <2.3** **Low: >=2.3 and <=3**		**Sum of scores:**			**30**	**53**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**2.208**	**Overall Score (Rounded):**	**2.2[1]**
		Overall Quality Level:		**Unacceptable[1]**		
Comment:	**Footnote:** **[1] Consistent with our *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one of the metrics was rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.**					

1.5 Animal toxicity evaluation results of Song et al 2016 for acute and 14-day inhalation-systemic toxicity study on body weight, hematological and immune, clinical chemistry/biochemical, hepatic, renal, respiratory, reproductive outcomes

Study reference:	**Song, N.,Li, L.,Li, H.,Ai, W.,Xie, W.,Yu, W.,Liu, W.,Wang, C.,Shen, G.,Zhou, L.,Wei, C.,Li, D.,Chen, H. (2016). Single and 14-day repeated dose inhalation toxicity studies of hexabromocyclododecane in rats Food and Chemical Toxicology, 91, 73-81** **HERO ID: 3350482**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium ,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance was clearly identified by name and CASRN.	High	1	2	2
	2. Test Substance Source	The test substance source/manufacturer was identified however the batch/lot number was not reported	Medium	2	1	2
	3. Test Substance Purity	The test substance purity was identified	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Negative control animals were included in the 14 day. No negative control required for acute study.	High	1	2	2
	5. Positive Controls	Positive controls not applicable.	Not Rated	NR	NR	NR
	6. Randomized Allocation	Animals were randomly allocated to each group.	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	The method and equipment used to generate the dust aerosol were reported and appropriate.	High	1	1	1
	8. Consistency of Exposure Administration	Exposures were administered consistently.	High	1	1	1
	9. Reporting of Doses/Concentrations	Target and measured concentrations, MMAD, and GSD were reported for all groups.	High	1	2	2
	10. Exposure Frequency and Duration	Frequency and duration were reported.	High	1	1	1

Study reference:	**Song, N.,Li, L.,Li, H.,Ai, W.,Xie, W.,Yu, W.,Liu, W.,Wang, C.,Shen, G.,Zhou, L.,Wei, C.,Li, D.,Chen, H. (2016). Single and 14-day repeated dose inhalation toxicity studies of hexabromocyclododecane in rats Food and Chemical Toxicology, 91, 73-81** **HERO ID: 3350482**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium ,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Number of Exposure Groups and Dose Spacing	The number of groups and spacing were reported along with rationale for concentration selection.	High	1	1	1
	12. Exposure Route and Method	The route and method were appropriate.	High	1	1	1
Test Organism	13. Test Animal Characteristics	The source, health status, species, strain, age, and sex were reported. Initial body weight was not reported.	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	All husbandry conditions were reported and appropriate.	High	1	1	1
	15. Number per Group	The number of animals per study group was appropriate.	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methodology was reported and appropriate.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling size was adequate.	High	1	1	1
	19. Blinding of Assessors	Blinding not required.	Not Rated	NR	NR	NR
	20. Negative Control Response	Negative control responses were appropriate.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	No confounding variables in test design were observed.	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	No health outcomes unrelated to exposure were reported.	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	Statistical methods were reported and appropriate.	High	1	1	1
	24. Reporting of Data	Data were reported.	High	1	2	2

Study reference:	Song, N.,Li, L.,Li, H.,Ai, W.,Xie, W.,Yu, W.,Liu, W.,Wang, C.,Shen, G.,Zhou, L.,Wei, C.,Li, D.,Chen, H. (2016). Single and 14-day repeated dose inhalation toxicity studies of hexabromocyclododecane in rats Food and Chemical Toxicology, 91, 73-81 **HERO ID: 3350482**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium ,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**32**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.1034**	**Overall Score: Nearest *:**	**1.1**
		Overall Quality Level:		**High**		

1.6 Animal toxicity evaluation results of Szabo et al 2016 for single gavage in mice on post-natal day 10; metabolomics evaluation only study on gene expression/omics outcomes

Study reference:	Szabo, D. T.,Pathmasiri, W.,Sumner, S.,Birnbaum, L. S. (2016). Serum Metabolomic Profiles in Neonatal Mice following Oral Brominated Flame Retardant Exposures to Hexabromocyclododecane (HBCD) Alpha, Gamma, and Commercial Mixture. Environmental Health Perspectives, 125(4), 651-659 **HERO ID: 3546063**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Chemical identity is clear; CAS #. provided Test substance is a commercial mixture of three stereoisomers. Percentages of each isomer are provided.	High	1	2	2
	2. Test Substance Source	Sourced from Sigma-Aldrich	High	1	1	1
	3. Test Substance Purity	Percentages of isomers in commercial mixture were provided.; it is not indicated whether other impurities are present, but the study authors indicate that chemicals were purchased at the highest purity level available. The authors did, however, go through a stereoisomer separation and thermal conversion process and it is not clear how pure the samples were after this process. Additionally, dosing solutions were made using corn oil and toluene that was evaporated under vacuum. Whether there was any remaining toluene is unknown, although all samples, including controls were treated equally.	Medium	2	1	2
Test Design	4. Negative and Vehicle Controls	Appropriate negative (vehicle) control was used.	High	1	2	2
	5. Positive Controls	Positive control not required.	Not Rated	NR	NR	NR

Study reference:	**Szabo, D. T.,Pathmasiri, W.,Sumner, S.,Birnbaum, L. S. (2016). Serum Metabolomic Profiles in Neonatal Mice following Oral Brominated Flame Retardant Exposures to Hexabromocyclododecane (HBCD) Alpha, Gamma, and Commercial Mixture. Environmental Health Perspectives, 125(4), 651-659** **HERO ID: 3546063**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	6. Randomized Allocation	Study does not indicate how dams and corresponding pups were allocated into treatment groups. Given the small number of total dams/litters (n = 7), and the fact that no statements are made indicating, for example, that dams and pup weights were equivalent, this introduces uncertainty that could impact results.	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	Study references previous publications for methods used for stereoisomer separation. Preparation of dosing solutions were appropriate. Since animals only received a single dose, storage of the dosing solutions was not necessary.	High	1	1	1
	8. Consistency of Exposure Administration	Dosing was equivalent across treatment groups (all animals given 10mg/kg gavage of appropriate treatment)	High	1	1	1
	9. Reporting of Doses/Concentrations	Doses were clearly stated	High	1	2	2
	10. Exposure Frequency and Duration	Single exposure via gavage	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	An explanation of chosen doses was provided	High	1	1	1
	12. Exposure Route and Method	Gavage was appropriate for pups that were still lactating, unclear whether 10ml/kg is appropriate though for pups that are PND10?	Medium	2	1	2

Study reference:	**Szabo, D. T.,Pathmasiri, W.,Sumner, S.,Birnbaum, L. S. (2016). Serum Metabolomic Profiles in Neonatal Mice following Oral Brominated Flame Retardant Exposures to Hexabromocyclododecane (HBCD) Alpha, Gamma, and Commercial Mixture. Environmental Health Perspectives, 125(4), 651-659** **HERO ID: 3546063**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Organism	13. Test Animal Characteristics	Study clearly explains reasoning for choosing mice at this stage of development	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Animal husbandry conditions were appropriate	High	1	1	1
	15. Number per Group	Study indicates that 6 female pups per litter (n = 7 litters total) were used for the experiment. Including the control, there is a total of 7 dose groups (control, 3-doses of alpha-HBCD, 2-doses of gamma HBCD, and a single dose of the commercial mixture). It is unclear how this would work, unless one litter was used exclusively as a control, and then 1 pup per litter (out of 6 remaining litters) received each treatment.? Overall, the total number of pups per treatment group is not explicitly stated and cannot be accurately inferred given the available data.	Low	3	1	3

Study reference:	**Szabo, D. T.,Pathmasiri, W.,Sumner, S.,Birnbaum, L. S. (2016). Serum Metabolomic Profiles in Neonatal Mice following Oral Brominated Flame Retardant Exposures to Hexabromocyclododecane (HBCD) Alpha, Gamma, and Commercial Mixture. Environmental Health Perspectives, 125(4), 651-659** **HERO ID: 3546063**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	16. Outcome Assessment Methodology	Metabolomic assessment of the blood was done via NMR at a single time-point (4-days post-exposure), which generally could miss key transitional changes. However, the study authors indicate that this time point was chosen to coincide with previous data collected from various tissues, and therefore seems appropriate. - NMR has relatively low sensitivity compared with other analytical tools for metabolomics, and no power analysis was done to determine an appropriate sample size. It is not clear whether technical replicates were included in the methodology.	Medium	2	2	4
	17. Consistency of Outcome Assessment	Outcome assessment appeared to be consistent across groups	High	1	1	1

Study reference:	**Szabo, D. T.,Pathmasiri, W.,Sumner, S.,Birnbaum, L. S. (2016). Serum Metabolomic Profiles in Neonatal Mice following Oral Brominated Flame Retardant Exposures to Hexabromocyclododecane (HBCD) Alpha, Gamma, and Commercial Mixture. Environmental Health Perspectives, 125(4), 651-659** **HERO ID: 3546063**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	18. Sampling Adequacy	Analysis was done on samples taken from 3 -6 pups/ treatment group. The number of control samples were not stated. It is unclear whether the differences in sample numbers across treatment groups was because those were the total number of animals treated, or whether for some reason, in some cases, samples were only collected from three out of 6 treated animals. Three biological replicates for an omics-based study is an absolute minimum and greatly reduces statistical power and has increased noise.	Low	3	1	3
	19. Blinding of Assessors	Blinding was not indicated, but not necessarily applicable to NMR analysis	Not Rated	NR	NR	NR
	20. Negative Control Response	The responses of the controls are presumed to be appropriate	High	1	1	1

Study reference:	**Szabo, D. T.,Pathmasiri, W.,Sumner, S.,Birnbaum, L. S. (2016). Serum Metabolomic Profiles in Neonatal Mice following Oral Brominated Flame Retardant Exposures to Hexabromocyclododecane (HBCD) Alpha, Gamma, and Commercial Mixture. Environmental Health Perspectives, 125(4), 651-659** **HERO ID: 3546063**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	The study authors do not discuss potential confounding variables. It is mentioned that there were no changes in body weights between treated and controls following treatment, but no statements were made indicating that the initial health and weights of treated pups were equivalent across litters leaving the potential for unknown confounding variables. There is also a potential for litter effects, however, this was presumably taken into account in the study design by treating across litters.	Low	3	2	6
	22. Health Outcomes Unrelated to Exposure	The study does not include observations (clinical or otherwise) of pups during or after dosing. It is still unclear why some treatment groups had three samples evaluated, and others had 6 samples evaluated, and whether this could potentially be due to problems with some of the animals, or if only three animals were treated.	Low	3	1	3
Data Presentation and Analysis	23. Statistical Methods	Statistical analysis was appropriate.	High	1	1	1
	24. Reporting of Data	Data presentation was adequate and appropriate for omics reporting. - Some data was presented in supplementary tables that were not available to view	High	1	2	2
		Sum of scores:			**29**	**45**

Study reference:	**Szabo, D. T.,Pathmasiri, W.,Sumner, S.,Birnbaum, L. S. (2016). Serum Metabolomic Profiles in Neonatal Mice following Oral Brominated Flame Retardant Exposures to Hexabromocyclododecane (HBCD) Alpha, Gamma, and Commercial Mixture. Environmental Health Perspectives, 125(4), 651-659** **HERO ID: 3546063**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium,Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
High: >=1 and <1.7 **Medium: >=1.7 and <2.3** **Low: >=2.3 and <=3**		**Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:**		**NR**	**Overall Score: Nearest *:**	**NR**
		Overall Quality Level:		**Medium**		
Study Quality Comment:	**The reviewer downgraded this study's overall quality rating. They noted: Problems with methods reporting (specifically the number of animals exposed/treatment group), as well as data indicating animals were of equivalent health and body weight at study initiation decrease confidence in the study results. Note: The original calculated score for this study was 1.5. This value is not presented above because the final rating was changed based on professional judgement.**					

2 Short-term Toxicity Studies

2.1 Animal toxicity evaluation results of Bernhard et al 2016 for 28-day oral exposure in mice via diet study on hepatic, body weight outcomes

Study reference:	**Bernhard, A.,Berntssen, M. H. G.,Lundebye, A. K.,Ra, Yneberg Alvheim, A.,Secher Myrmel, L.,Fja, Re, E.,Torstensen, B. E.,Kristiansen, K.,Madsen, L.,Brattelid, T.,Rasinger, J. D. (2016). Marine fatty acids aggravate hepatotoxicity of HBCD in juvenile female BALB/c mice, 97, 411-423** **HERO ID: 3588138**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Identity and form are stated, no CAS# reported.	High	1	2	2
	2. Test Substance Source	alpha-HBCD was synthesized from from gamma-HBCD. Analytical verification of the product was not done, however, concentrations in feed were analyzed by GC-MS.	Medium	2	1	2
	3. Test Substance Purity	After production, purity of the alpha isomer was described as "pure". alpha-HBCD was produced in the laboratory. Study report states that "purified alpha-HBCD" was used to dose animals but % purity or details on the purification methods were not provided.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Study used an appropriate vehicle negative control diet.	High	1	2	2
	5. Positive Controls	Positive control not necessary	Not Rated	NR	NR	NR
	6. Randomized Allocation	It was stated that animals were randomly assigned, although the method for assignment was not described.	Medium	2	1	2

Study reference:	**Bernhard, A.,Berntssen, M. H. G.,Lundebye, A. K.,Ra, Yneberg Alvheim, A.,Secher Myrmel, L.,Fja, Re, E.,Torstensen, B. E.,Kristiansen, K.,Madsen, L.,Brattelid, T.,Rasinger, J. D. (2016). Marine fatty acids aggravate hepatotoxicity of HBCD in juvenile female BALB/c mice, 97, 411-423** **HERO ID: 3588138**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Exposure Characterization	7. Preparation and Storage of Test Substance	The frequency of diet preparation and a statement about stability were not provided. Preparation of diets was acceptable.	Medium	2	1	2
	8. Consistency of Exposure Administration	administration was consistent across groups.	High	1	1	1
	9. Reporting of Doses/Concentrations	Both nominal and measured concentrations in the diet were provided with corresponding daily exposures. However, these values were calculated using estimated (rather than actual) daily food intake. It can not be determined whether there was a difference in the intake across treatment groups.	Low	3	2	6
	10. Exposure Frequency and Duration	Appropriate; study design was based on OECD guideline 407 for short-term repeated dose toxicity study	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	Number of exposure groups was appropriate. Authors state that "The high dose (HD) chosen was high enough to elicit molecular aberrations and the low dose (LD) was based on the potentially relevant Lowest Observed Adverse Effect Level (LOAEL) (Table 1; Yanagisawa et al., 2014)."	High	1	1	1
	12. Exposure Route and Method	Exposure route acceptable	High	1	1	1

Study reference:	**Bernhard, A.,Berntssen, M. H. G.,Lundebye, A. K.,Ra, Yneberg Alvheim, A.,Secher Myrmel, L.,Fja, Re, E.,Torstensen, B. E.,Kristiansen, K.,Madsen, L.,Brattelid, T.,Rasinger, J. D. (2016). Marine fatty acids aggravate hepatotoxicity of HBCD in juvenile female BALB/c mice, 97, 411-423** **HERO ID: 3588138**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Organism	13. Test Animal Characteristics	Standard animal model was used. Age was appropriate for desired "juvenile" developmental time point. Only one sex evaluated. Animals were obtained from Taconic.	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Animal husbandry clearly reported and appropriate.	High	1	1	1
	15. Number per Group	n = 3-8 / group, depending on the outcome evaluated. Sample size is below the recommended minimum (n = 10) for OECD 407.	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	Methodology of outcome assessments were clearly described and appropriate	High	1	2	2
	17. Consistency of Outcome Assessment	Consistent assessment across groups.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate. Histology was performed on a subset of animals (n=3-4) from each exposure group, including controls	High	1	1	1
	19. Blinding of Assessors	Histopathology evaluations were subjective. Study report does not indicate that the assessor was blinded during assessment or whether outcomes were evaluated independently by a second pathologist.	Medium	2	1	2
	20. Negative Control Response	No out of the ordinary control responses were noted.	High	1	1	1

Study reference:	**Bernhard, A.,Berntssen, M. H. G.,Lundebye, A. K.,Ra, Yneberg Alvheim, A.,Secher Myrmel, L.,Fja, Re, E.,Torstensen, B. E.,Kristiansen, K.,Madsen, L.,Brattelid, T.,Rasinger, J. D. (2016). Marine fatty acids aggravate hepatotoxicity of HBCD in juvenile female BALB/c mice, 97, 411-423** **HERO ID: 3588138**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	Initial body weights of animals were not reported. It is unclear whether there were differences in feed consumption because a default value (15% w/w) was used rather than the actual dietary intake	Low	3	2	6
	22. Health Outcomes Unrelated to Exposure	No health outcomes unrelated to exposure were reported; animals were observed daily.	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	Statistical analysis methodology were clearly reported and appropriate.	High	1	1	1
	24. Reporting of Data	Reporting of data was appropriate for most outcomes. Confidence level for histopathology results is reduced to Medium because results are only presented qualitatively (representative histology images from each group were shown and text description of the effects).	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**45**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**NR**	**Overall Score: Nearest *:**	**NR**
		Overall Quality Level:		**Medium**		
Study Quality Comment:	**The reviewer downgraded this study's overall quality rating. They noted: I would downgrade this study based on concerns related to the purity of the chemical and reporting of the doses/concentrations. Note: The original calculated score for this study was 1.5. This value is not presented above because the final rating was changed based on professional judgement.**					

2.2 Animal toxicity evaluation results of Genskow et al 2015 for 30 day oral toxicity study (daily gavage); primarily mechanistic, also contains in vitro data study on neurological/behavior outcomes

Study reference:	**Genskow, K. R.,Bradner, J. M.,Hossain, M. M.,Richardson, J. R.,Caudle, W. M. (2015). Selective damage to dopaminergic transporters following exposure to the brominated flame retardant, HBCDD Neurotoxicology and Teratology, 52(Pt B), 162-169** **HERO ID: 2919804**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance name was provided but CAS# was not provided	Medium	2	2	4
	2. Test Substance Source	Test substance source was provided but batch or lot number was not reported	Medium	2	1	2
	3. Test Substance Purity	Purity of the test substance is not reported	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Vehicle control reported	High	1	2	2
	5. Positive Controls	A positive control was not necessary, but could have provided useful information in this study that would aid in the interpretation of the results	Not Rated	NR	NR	NR
	6. Randomized Allocation	The study does not indicate whether animals were randomized, the endpoints evaluated were more mechanistic in nature, and may not have been impacted greatly by randomization.	Medium	2	1	2
Exposure Characterization	7. Preparation and Storage of Test Substance	Details of preparation, frequency of preparation, and storage were lacking	Low	3	1	3
	8. Consistency of Exposure Administration	Control and treatment groups were treated consistently	High	1	1	1
	9. Reporting of Doses/Concentrations	Dose concentrations were clearly reported, however, no validation of dose was performed by the study authors.	Medium	2	2	4
	10. Exposure Frequency and Duration	Exposure frequency and duration were clearly reported	High	1	1	1

Study reference:	**Genskow, K. R.,Bradner, J. M.,Hossain, M. M.,Richardson, J. R.,Caudle, W. M. (2015). Selective damage to dopaminergic transporters following exposure to the brominated flame retardant, HBCDD Neurotoxicology and Teratology, 52(Pt B), 162-169** **HERO ID: 2919804**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Number of Exposure Groups and Dose Spacing	Single dose exposure that did not induce effects for several endpoints measured. It is unclear whether HBCD indeed has no effect, or whether a dose-limit was not reached NK: Single dose exposure, daily for 30 days. Control had 4 mice and treatment group had 6 mice.	Medium	2	1	2
	12. Exposure Route and Method	Exposure route and method were acceptable.	High	1	1	1
Test Organism	13. Test Animal Characteristics	Animals (C57BL/6 male mice) were purchased at 8weeks old and the mice were treated when they were 3 months old (4 weeks later). Animals are generally acclimatized for a week; 4 weeks seems a bit odd.	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	Animal husbandry details were not provided, but the study authors state that procedures were conducted in accordance with the guide for care and use of laboratory animals	Medium	2	1	2
	15. Number per Group	Four control animals and 6 treated animals of a single sex were used. OECD guidelines for 28-day toxicity studies recommends an n of 10 (5 animals of each sex).	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	The outcome assessment methodology addressed or reported the intended outcome(s) of interest and was sensitive for the outcome(s) of interest.	High	1	2	2

Study reference:	**Genskow, K. R.,Bradner, J. M.,Hossain, M. M.,Richardson, J. R.,Caudle, W. M. (2015). Selective damage to dopaminergic transporters following exposure to the brominated flame retardant, HBCDD Neurotoxicology and Teratology, 52(Pt B), 162-169** **HERO ID: 2919804**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	17. Consistency of Outcome Assessment	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups	High	1	1	1
	18. Sampling Adequacy	The study reported adequate sampling for the outcome(s) of interest	High	1	1	1
	19. Blinding of Assessors	Blinding is not required for this methodology	Not Rated	NR	NR	NR
	20. Negative Control Response	Control responses appear to be appropriate	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	No confounding variables were noted, however, data regarding other potential exposure-related effects (i.e,, potential effects on body weight), were not included in the report.	Medium	2	2	4
	22. Health Outcomes Unrelated to Exposure	This information was not included in the study report or in the study design.	Medium	2	1	2
Data Presentation and Analysis	23. Statistical Methods	Statistical analysis was acceptable	High	1	1	1
	24. Reporting of Data	Reporting of data (for the methods used) was acceptable.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**47**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**NR**	**Overall Score: Nearest *:**	**NR**
		Overall Quality Level:		**Medium**		
Study Quality Comment:	**The reviewer downgraded this study's overall quality rating. They noted: Downgraded the study from 'high' to 'medium' because this is primarily a mechanistic study. The small part of the study that is animal toxicity study with just one dose and has fewer animals (n=4 for control) and n=6 for treatment group). Note: The original calculated score for this study was 1.6. This value is not presented above because the final rating was changed based on professional judgement.**					

2.3 Animal toxicity evaluation results of Hachisuka et al 2010 for oral developmental immunotoxicity study on hematological and immune outcomes

Study reference:	**Hachisuka, A.,Nakamura, R.,Sato, Y.,Nakamura, R.,Shibutani, M.,Teshima, R. (2010). [Effects of perinatal exposure to the brominated flame-retardant hexabromocyclododecane (HBCD) on the developing immune system in rats] Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku, [2010](128), 58-64** **HERO ID: 1403765**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by name.	Medium	2	2	4
	2. Test Substance Source	Source not identified.	Low	3	1	3
	3. Test Substance Purity	Composition and purity not reported.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Concurrent negative control animals are included.	High	1	2	2
	5. Positive Controls	Positive controls not required.	Not Rated	NR	NR	NR
	6. Randomized Allocation	Allocation methods were not reported.	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	Limited details on preparation (mixed into the food) and no information on storage and stability were reported.	Low	3	1	3
	8. Consistency of Exposure Administration	Animals were allowed to feed freely on the diet, but no details on the amount of diet provided was reported.	Medium	2	1	2
	9. Reporting of Doses/Concentrations	Concentrations were reported.	High	1	2	2
	10. Exposure Frequency and Duration	Exposure duration was reported.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	The number of exposure groups and spacing were reported, but not justified.	Medium	2	1	2
	12. Exposure Route and Method	The exposure route and method were appropriate.	High	1	1	1

Study reference:	**Hachisuka, A.,Nakamura, R.,Sato, Y.,Nakamura, R.,Shibutani, M.,Teshima, R. (2010). [Effects of perinatal exposure to the brominated flame-retardant hexabromocyclododecane (HBCD) on the developing immune system in rats] Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku, [2010](128), 58-64** **HERO ID: 1403765**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Organism	13. Test Animal Characteristics	The species, strain, and sex were reported. The source and starting body weight of dams were not reported.	Low	3	2	6
	14. Adequacy and Consistency of Animal Husbandry Conditions	Details were not reported.	Low	3	1	3
	15. Number per Group	The number of animals per group was appropriate.	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methodology was reported for some outcomes- hematology, thymus and spleen weight and pathology, and immunity. Other outcomes assessment methodology, including body weight and weight gain, were not reported.	Medium	2	2	4
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling for some outcomes was not reported or illegible.	Medium	2	1	2
	19. Blinding of Assessors	Blinding not required.	Not Rated	NR	NR	NR
	20. Negative Control Response	Negative control responses were appropriate.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	Initial body weight and food/water intake of same were not reported and appear not to have been measured.	Low	3	2	6
	22. Health Outcomes Unrelated to Exposure	There were not reported differences among the groups in health outcomes unrelated to exposures.	High	1	1	1

Study reference:	Hachisuka, A.,Nakamura, R.,Sato, Y.,Nakamura, R.,Shibutani, M.,Teshima, R. (2010). [Effects of perinatal exposure to the brominated flame-retardant hexabromocyclododecane (HBCD) on the developing immune system in rats] Kokuritsu Iyakuhin Shokuhin Eisei Kenkyusho Hokoku, [2010](128), 58-64 **HERO ID: 1403765**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Data Presentation and Analysis	23. Statistical Methods	Statistical methods were not described but were conducted, and data were provided to conduct an independent analysis.	Medium	2	1	2
	24. Reporting of Data	Data were reported by groups, however it appears that not all outcomes were reported by sex.	Medium	2	2	4
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**57**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.9655**	**Overall Score: Nearest *:**	**2**
		Overall Quality Level:		**Medium**		

2.4 Animal toxicity evaluation results of Maranghi et al 2013 for 28-day dietary study on hepatic, body weight, thyroid, hematological and immune, reproductive outcomes

Study reference:	**Maranghi, F.,Tassinari, R.,Moracci, G.,Altieri, I.,Rasinger, J. D.,Carroll, T. S.,Hogstrand, C.,Lundebye, A. K.,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 and Chemical Toxicology, 56, 443-449** **HERO ID: 1927558**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Chemical name provided, no CAS #, and no structure provided.	Medium	2	2	4
	2. Test Substance Source	The source was no reported, no verification or analytical assessment	Low	3	1	3
	3. Test Substance Purity	Substance purity was not provided	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	An appropriate negative control was used	High	1	2	2
	5. Positive Controls	Positive control was not required	Not Rated	NR	NR	NR
	6. Randomized Allocation	Mice were allocated at random; method used was not detailed	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Preparation of exposure diets were described, however the frequency of preparation and details of storage were not indicated.	Medium	2	1	2
	8. Consistency of Exposure Administration	Exposure was consistent across groups. - Animals were restricted to 15% w/w food intake.	High	1	1	1

Study reference:	**Maranghi, F.,Tassinari, R.,Moracci, G.,Altieri, I.,Rasinger, J. D.,Carroll, T. S.,Hogstrand, C.,Lundebye, A. K.,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 and Chemical Toxicology, 56, 443-449** **HERO ID: 1927558**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	9. Reporting of Doses/Concentrations	Do to methodological limitations, the intended HBCD concentration in feed could not be verified. It was therefore presumed that the concentration was equivalent to the intended dose. Analysis of other chemicals evaluated in the same study, indicated they were essentially the same as the intended inclusion levels.	Medium	2	2	4
	10. Exposure Frequency and Duration	Frequency and duration were clearly reported	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	Single dose and a control. - Justification of dose was provided.	High	1	1	1
	12. Exposure Route and Method	Exposure route and method was acceptable	High	1	1	1
Test Organism	13. Test Animal Characteristics	Appropriate test organism	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Animal husbandry acceptable	High	1	1	1
	15. Number per Group	15/control group 10/treatment group	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Methods of outcome assessment were appropriate.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently across groups	High	1	1	1
	18. Sampling Adequacy	Sampling sizes were adequate	High	1	1	1
	19. Blinding of Assessors	Blinding of assessors was not reported but is not required for initial histology evaluation.	Medium	2	1	2

Study reference:	**Maranghi, F.,Tassinari, R.,Moracci, G.,Altieri, I.,Rasinger, J. D.,Carroll, T. S.,Hogstrand, C.,Lundebye, A. K.,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 and Chemical Toxicology, 56, 443-449** **HERO ID: 1927558**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	20. Negative Control Response	No abnormal control responses were reported	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	No confounding variables were identified.	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	There were no unrelated exposure health outcomes	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	Appropriate statistical methods were utilized	High	1	1	1
	24. Reporting of Data	Data reporting was acceptable	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**40**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.3333**	**Overall Score: Nearest *:**	**1.3**
		Overall Quality Level:		**High**		

2.5 Animal toxicity evaluation results of Miller et al 2016 for mechanism of liver and thyroid toxicity study on hepatic, thyroid outcomes

Study reference:	**Miller, I.,Serchi, T.,Cambier, S.,Diepenbroek, C.,Renaut, J.,Van der Berg, J. H.,Kwadijk, C.,Gutleb, A. C.,Rijntjes, E.,Murk, A. J. (2016). Hexabromocyclododecane (HBCD) induced changes in the liver proteome of eu- and hypothyroid female rats Toxicology Letters, 245, 40-51** **HERO ID: 3350495**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by name. No CAS # or other details were provided	Medium	2	2	4
	2. Test Substance Source	Source or manufacturer was not identified.	Low	3	1	3
	3. Test Substance Purity	Purity of the substance was not provided	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Concurrent negative controls were included.	High	1	2	2
	5. Positive Controls	Positive controls were not required.	Not Rated	NR	NR	NR
	6. Randomized Allocation	Allocation methods were not reported.	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	Preparation of the test substance was reported but storage prior to administration was not reported.	Medium	2	1	2
	8. Consistency of Exposure Administration	Exposures were administered consistently.	High	1	1	1
	9. Reporting of Doses/Concentrations	Appropriate doses were reported	High	1	2	2
	10. Exposure Frequency and Duration	Frequency and duration were reported.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	The number of groups and spacing were reported	High	1	1	1
	12. Exposure Route and Method	The route and method were appropriate.	High	1	1	1

Study reference:	**Miller, I.,Serchi, T.,Cambier, S.,Diepenbroek, C.,Renaut, J.,Van der Berg, J. H.,Kwadijk, C.,Gutleb, A. C.,Rijntjes, E.,Murk, A. J. (2016). Hexabromocyclododecane (HBCD) induced changes in the liver proteome of eu- and hypothyroid female rats Toxicology Letters, 245, 40-51** **HERO ID: 3350495**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Organism	13. Test Animal Characteristics	The source, species, strain, and age were reported. Initial body weight was not reported. Some animals were iodine depleted to create a hypothyroid state resulting in 2 groups, normal and hypothyroid.	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	The temperature, humidity, lighting, water, and diet were reported. No other details were reported.	Medium	2	1	2
	15. Number per Group	The number of animals per group was appropriate.	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methodology was reported and appropriate.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate.	High	1	1	1
	19. Blinding of Assessors	Blinding was not required.	Not Rated	NR	NR	NR
	20. Negative Control Response	Negative control responses were appropriate.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	Iodine depletion may have an effect on the results	Medium	2	2	4
	22. Health Outcomes Unrelated to Exposure	One group of animals were exposed in a hypothyroid state.	Medium	2	1	2
Data Presentation and Analysis	23. Statistical Methods	Statistical methods were reported and appropriate.	High	1	1	1
	24. Reporting of Data	Data were reported.	High	1	2	2
		Sum of scores:			**29**	**44**

Study reference:	**Miller, I.,Serchi, T.,Cambier, S.,Diepenbroek, C.,Renaut, J.,Van der Berg, J. H.,Kwadijk, C.,Gutleb, A. C.,Rijntjes, E.,Murk, A. J. (2016). Hexabromocyclododecane (HBCD) induced changes in the liver proteome of eu- and hypothyroid female rats Toxicology Letters, 245, 40-51** **HERO ID: 3350495**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:**		**NR**	**Overall Score: Nearest *:**	**NR**
		Overall Quality Level:		**Medium**		
Study Quality Comment:	**The reviewer downgraded this study's overall quality rating. They noted: This seem to be a well conducted study, however, one major flaw is that the source of HBCD was not reported. Not sure if the chemical was prepared in the lab or purchased from a manufacturer. Left the rating for metric 2 as low, but could be changed to unacceptable since information on test material source, manufacturer, purity, other analytical details of HBCD was not provided. Other parts of the study were appropriately conducted. Note: The original calculated score for this study was 1.5. This value is not presented above because the final rating was changed based on professional judgement.**					

2.6 Animal toxicity evaluation results of Miller-Rhodes et al 2014 for developmental study; gestation day 1-parturition study on growth (early life) and development, neurological/behavior outcomes

Study reference:	**Miller-Rhodes, P.,Popescu, M.,Goeke, C.,Tirabassi, T.,Johnson, L.,Markowski, V. P. (2014). Prenatal exposure to the brominated flame retardant hexabromocyclododecane (HBCD) impairs measures of sustained attention and increases age-related morbidity in the Long-Evans rat Neurotoxicology and Teratology, 45, 34-43** **HERO ID: 2528337**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Name and product number provided	High	1	2	2
	2. Test Substance Source	Commercial source	High	1	1	1
	3. Test Substance Purity	Purity >95%	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Use of vehicle control	High	1	2	2
	5. Positive Controls	Positive control not necessary	Not Rated	NR	NR	NR
	6. Randomized Allocation	Randomized block design	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Prepared fresh daily, properly mixed.	High	1	1	1
	8. Consistency of Exposure Administration	Exposure consistent across groups	High	1	1	1
	9. Reporting of Doses/Concentrations	concentrations were reported	High	1	2	2
	10. Exposure Frequency and Duration	Daily gavage	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	Three dose groups and a control	High	1	1	1
	12. Exposure Route and Method	Gavage	High	1	1	1
Test Organism	13. Test Animal Characteristics	Standard animal model used (Long Evans rats)	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Animal husbandry was reported and acceptable	High	1	1	1

Study reference:	**Miller-Rhodes, P.,Popescu, M.,Goeke, C.,Tirabassi, T.,Johnson, L.,Markowski, V. P. (2014). Prenatal exposure to the brominated flame retardant hexabromocyclododecane (HBCD) impairs measures of sustained attention and increases age-related morbidity in the Long-Evans rat Neurotoxicology and Teratology, 45, 34-43** **HERO ID: 2528337**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	10-11 pregnant dams/treatment group. (litters culled to 8 pups using randomized selection procedure)	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methods were appropriate	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently across groups	High	1	1	1
	18. Sampling Adequacy	It is unclear the number of animals evaluated for each outcome. The "n" is consistently stated. Although it was mentioned that litters were culled to 8 pups, there were a number of deaths, so it is not clear how many were left for further analysis. It is stated that every pup in each litter was examined, for example, for FOB tests, but it is not known what differences in n there is between exposure groups, or if there are any. In some cases, it is mentioned that one male and one female from each litter were used for some endpoints, but it is not clear this was always the case.	Low	3	1	3
	19. Blinding of Assessors	Stated that observers were blind to the exposure group	High	1	1	1

Study reference:	**Miller-Rhodes, P.,Popescu, M.,Goeke, C.,Tirabassi, T.,Johnson, L.,Markowski, V. P. (2014). Prenatal exposure to the brominated flame retardant hexabromocyclododecane (HBCD) impairs measures of sustained attention and increases age-related morbidity in the Long-Evans rat Neurotoxicology and Teratology, 45, 34-43** **HERO ID: 2528337**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	20. Negative Control Response	Study authors indicate that the mean gestation length of the control group was shorter than typically expected for these rats, which may be the reason why HBCD treated rats appeared to have a longer gestation period.	Medium	2	1	2
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	Study authors mention that the ability to detect an exposure effect for locomotor activity could have been confounded by different body size to chamber size ratios. It was also mentioned that paw sizes were not taken into account for the grip strength tests	Medium	2	2	4
	22. Health Outcomes Unrelated to Exposure	There were a number of animals that disproportionately died unexpectedly or became ill. The authors indicate that data from these animals were not used for several of the analyses. Since the actual numbers of animals effected were not reported, it is unclear how this impacted the analyses or the actual number of animals evaluated for each endpoint. The timing of when these animals died or became ill is also not reported.	Low	3	1	3

Study reference:	**Miller-Rhodes, P.,Popescu, M.,Goeke, C.,Tirabassi, T.,Johnson, L.,Markowski, V. P. (2014). Prenatal exposure to the brominated flame retardant hexabromocyclododecane (HBCD) impairs measures of sustained attention and increases age-related morbidity in the Long-Evans rat Neurotoxicology and Teratology, 45, 34-43** **HERO ID: 2528337**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Data Presentation and Analysis	23. Statistical Methods	The described statistical analysis was appropriate, and the litter was used as the unit of analysis for offspring endpoints, however, results from statistical analysis were not shown in any of the figures making it difficult to easily interpret the data. In most instances, p-values were provided within the text.	Medium	2	1	2
	24. Reporting of Data	No individual offspring animal data were reported, therefore the data cannot be independently reviewed. Additionally, most data are reported in the form of bar graphs, and text does not provide the quantal values. Data from males and females were often pooled and averaged, and therefore not reported independently.	Low	3	2	6
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**42**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**NR**	**Overall Score: Nearest *:**	**NR**
		Overall Quality Level:		**Medium**		
Study Quality Comment:	**The reviewer downgraded this study's overall quality rating. They noted: The lack of individual animal data, and the way the data is presented, make it difficult to interpret the data. Additionally, the lack of clarity regarding the number of animals evaluated should be considered. There were also a large number of animals that became ill. Without further transparency or information, it is difficult to know how this could have impacted the various results with the data provided Note: The original calculated score for this study was 1.4. This value is not presented above because the final rating was changed based on professional judgement.**					

2.7 Animal toxicity evaluation results of van et al 2006 for 280day oral toxicity study (gavage) study on hepatic, clinical chemistry/biochemical, endocrine, musculoskeletal/motor function, ADME/PBPK, thyroid, nutrition and metabolic/adult exposure body weight, hematological and immune, reproductive outcomes

Study reference:	**van der Ven, L. T.,Verhoef, A.,van de Kuil, T.,Slob, W.,Leonards, P. E.,Visser, T. J.,Hamers, T.,Herlin, M.,Håkansson, H.,Olausson, H.,Piersma, A. H.,Vos, J. G. (2006). A 28-day oral dose toxicity study enhanced to detect endocrine effects of hexabromocyclododecane in Wistar rats Toxicological Sciences, 94(2), 281-292** **HERO ID: 787745**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	The test substance was identified definitively and characterized. HBCD technical preparation is a mixture of three enantiomers, HBCD-alpha- beta-, and gamma, and their respective proportion in the used batch was 10.28, 8.72, and 81.01%, respectively.	High	1	2	2
	2. Test Substance Source	The source (manufacturer) of the test substance was reported, but the batch/lot numbers were omitted; this omission is unlikely to have a substantial impact on results.	Medium	2	1	2
	3. Test Substance Purity	The test substance was noted to be technical HBCD as a mixture of three enantiomers, HBCD-alpha- beta-, and gamma, with respective proportions as 10.28, 8.72, and 81.01%, respectively. Trace impurities were identified as traces of tetra- and pentabromocyclododecane.	High	1	1	1
Test Design	4. Negative and Vehicle Controls	An appropriate concurrent negative control group was included.	High	1	2	2

Study reference:	van der Ven, L. T.,Verhoef, A.,van de Kuil, T.,Slob, W.,Leonards, P. E.,Visser, T. J.,Hamers, T.,Herlin, M.,Håkansson, H.,Olausson, H.,Piersma, A. H.,Vos, J. G. (2006). A 28-day oral dose toxicity study enhanced to detect endocrine effects of hexabromocyclododecane in Wistar rats Toxicological Sciences, 94(2), 281-292 **HERO ID: 787745**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	5. Positive Controls	The use of a positive control was reported for the UDP-glucuronosyltransferase assay. This metric was not rated/applicable for the other evaluations in the study.	Medium	2	1	2
	6. Randomized Allocation	"The experimental protocol followed the OECD407 28-day sub-acute toxicity guideline, which was enhanced for endocrine and immunological endpoints (Andrews et al., 2001). However, in contrast to the published protocol, the animals were distributed among more dose groups each with fewer animals, that is, five rats per sex per dose group, for improved assessment of dose response relationships (Kavlock et al., 1996; Slob, 2002)." It is unclear if this would have a substantial impact on results.	Medium	2	1	2
Exposure Characterization	7. Preparation and Storage of Test Substance	Test substance preparation was reported, but with limitations in reporting. HBCD was reported to be dissolved in corn oil. It is not reported how often the test solution was prepared or how it was stored. This omission is unlikely to have a substantial impact on results.	Medium	2	1	2

Study reference:	van der Ven, L. T.,Verhoef, A.,van de Kuil, T.,Slob, W.,Leonards, P. E.,Visser, T. J.,Hamers, T.,Herlin, M.,Håkansson, H.,Olausson, H.,Piersma, A. H.,Vos, J. G. (2006). A 28-day oral dose toxicity study enhanced to detect endocrine effects of hexabromocyclododecane in Wistar rats Toxicological Sciences, 94(2), 281-292 **HERO ID: 787745**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	8. Consistency of Exposure Administration	Details of exposure administration were reported and administration was consistent across study groups.	High	1	1	1
	9. Reporting of Doses/Concentrations	Administered doses were reported without ambiguity.	High	1	2	2
	10. Exposure Frequency and Duration	The exposure frequency and duration of exposure were reported and appropriate for this study type and/or outcome(s) of interest.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	The number of exposure groups and spacing was reported. It was reported that a larger number of dose groups was used (than recommended in OECD 407) for improved assessment of the dose-response relationship.	High	1	1	1
	12. Exposure Route and Method	The route and method of exposure were reported and were suited to the test substance.	High	1	1	1
Test Organism	13. Test Animal Characteristics	The test animal species, strain, sex, and age were reported. It was noted that the animals were inspected daily for general condition and clinical abnormalities. The animals were obtained from a commercial breeding facility.	High	1	2	2

Study reference:	van der Ven, L. T.,Verhoef, A.,van de Kuil, T.,Slob, W.,Leonards, P. E.,Visser, T. J.,Hamers, T.,Herlin, M.,Håkansson, H.,Olausson, H.,Piersma, A. H.,Vos, J. G. (2006). A 28-day oral dose toxicity study enhanced to detect endocrine effects of hexabromocyclododecane in Wistar rats Toxicological Sciences, 94(2), 281-292 **HERO ID: 787745**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	14. Adequacy and Consistency of Animal Husbandry Conditions	Most animal husbandry conditions were reported and adequate. Humidity and temperature were not reported, however, this limitation in reporting is unlikely to have a substantial impact on results.	Medium	2	1	2
	15. Number per Group	The number of animals per study group was reported (5/sex/dose). OECD 407 requires at least 10 animals (5/sex) for each dose level. Hence, the confidence is selected as 'medium'.	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	The outcome assessment methodology reported and sensitive to the intended outcomes of interest.	High	1	2	2
	17. Consistency of Outcome Assessment	Details of the outcome assessment methodology were reported and consistent across study groups for the outcomes of interest.	High	1	1	1
	18. Sampling Adequacy	Details regarding the sampling for the outcomes of interest were reported and adequate for assessment.	High	1	1	1
	19. Blinding of Assessors	This metric is not rated when outcomes are not subjective or for initial histopathology review.	Not Rated	NR	NR	NR
	20. Negative Control Response	The biological response of the negative control group was adequate. As shown in Data tables and in Supplemental tables (ID2919527)	High	1	1	1

Study reference:	van der Ven, L. T.,Verhoef, A.,van de Kuil, T.,Slob, W.,Leonards, P. E.,Visser, T. J.,Hamers, T.,Herlin, M.,Håkansson, H.,Olausson, H.,Piersma, A. H.,Vos, J. G. (2006). A 28-day oral dose toxicity study enhanced to detect endocrine effects of hexabromocyclododecane in Wistar rats Toxicological Sciences, 94(2), 281-292 **HERO ID: 787745**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	There were no reported differences among the study groups that could influence the outcome of the assessment. Food consumption was reported, but initial body weights were not. The lack of reporting is not likely to have a significant impact on results.	Medium	2	2	4
	22. Health Outcomes Unrelated to Exposure	Data on attrition unrelated to exposure was reported. No other health outcomes unrelated to exposure were reported. The incidence of attrition is unlikely to have a substantial impact on results.	Medium	2	1	2
Data Presentation and Analysis	23. Statistical Methods	Statistical analysis was shown for all datasets included in the published report and for supplemental data tables (ID2919527). BMD methodology was clearly described and appropriate.	High	1	1	1
	24. Reporting of Data	Data for exposure-related findings were presented for all outcomes by exposure group and sex as evaluated for this reference and the supplemental data tables (ID2919527).	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**39**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.3**	**Overall Score: Nearest *:**	**1.3**
		Overall Quality Level:		**High**		

2.8 Animal toxicity evaluation results of W. I. L. Research 1997 for 28-day repeated oral study on mortality, nutrition and metabolic/adult exposure body weight, neurological/behavior, hematological and immune, clinical chemistry/biochemical, hepatic, renal, cardiovascular, reproductive, endocrine, gastrointestinal, respiratory outcomes

Study reference:	**W. I. L. Research (1997). Twenty-eight day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 3/18/1997** **HERO ID: 787758**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	The test substance was identified definitively.	High	1	2	2
	2. Test Substance Source	The source of the test substance was reported, including manufacturer and lot number.	High	1	1	1
	3. Test Substance Purity	The study authors stated that the purity was "considered to be 100%", but no verification of this purity was reported.	Medium	2	1	2
Test Design	4. Negative and Vehicle Controls	The study authors reported using an appropriate concurrent negative control group (administered the vehicle via gavage at the same dose volume).	High	1	2	2
	5. Positive Controls	Positive control is not indicated by study type.	Not Rated	NR	NR	NR
	6. Randomized Allocation	The study reported methods of allocation of animals to study groups, but there were minor limitations in the allocation method (method of distribution had a non-random component, including assignment to minimize differences in body weight across groups).	Medium	2	1	2

Study reference:	**W. I. L. Research (1997). Twenty-eight day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 3/18/1997** **HERO ID: 787758**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Exposure Characterization	7. Preparation and Storage of Test Substance	The test substance preparation and storage conditions were reported and appropriate for the test substance (the test substance was prepared daily and stored at room temperature). Storage of the bulk test substance was also reported (sealed container at room temperature) and the bulk test substance was considered stable under the storage conditions.	High	1	1	1
	8. Consistency of Exposure Administration	Details of the administration were reported but minor limitations in administration of the exposures, including accidental mistakes in dosing, were identified that are unlikely to have a substantial impact on results. On one particular day, animals at higher dose levels were inadvertently dosed with lower doses, and a few lower dose animals were inadvertently dosed with higher doses. Lower doses were corrected so that the underdosed animals received the correct doses.	Medium	2	1	2
	9. Reporting of Doses/Concentrations	Administered doses were reported without ambiguity. Test concentrations were evaluated by gravimetric analysis each day prior to dosing and homogeneity was evaluated on three days during the administration period (d 0, 13, 27); however, the results were not reported.	Medium	2	2	4

Study reference:	W. I. L. Research (1997). Twenty-eight day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 3/18/1997 **HERO ID: 787758**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	10. Exposure Frequency and Duration	The exposure frequency and duration of exposure (daily exposure for 28 consecutive days) were reported and appropriate for the study type and outcomes of interest.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	The number of exposure groups and dose spacing (125, 350, 1000 mg/kg/day) were considered adequate to address the purpose of the study. Although the basis for selection of the doses was not reported, the range of doses was adequate.	High	1	1	1
	12. Exposure Route and Method	The route and method of exposure (oral, gavage) were reported and were suited to the test substance.	High	1	1	1
Test Organism	13. Test Animal Characteristics	The test animal source, species, strain, sex, age, and starting body weight (group means) were reported; however, health status was not reported.	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	All husbandry conditions (temperature, humidity, light-dark cycle) were reported and were adequate and the same for control and exposed populations.	High	1	1	1

Study reference:	W. I. L. Research (1997). Twenty-eight day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 3/18/1997 **HERO ID: 787758**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	The reported number of animals was lower than the typical number used in studies of the same or similar type for some groups; however, the number was sufficient for statistical analysis. The low- and mid-dose groups had only 6/sex/group, while the control and high-dose groups had 12/sex/group (6/sex/group sacrificed at the end of the 28-day administration period and the remaining 6/sex/group were maintained for an additional 14-day recovery period).	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	The outcome assessment methodology addressed or reported the intended outcomes of interest and was sensitive for the outcomes of interest.	High	1	2	2
	17. Consistency of Outcome Assessment	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.	High	1	1	1
	18. Sampling Adequacy	Details regarding the sampling for the outcomes of interest were reported and the study used adequate sampling for the outcomes of interest.	High	1	1	1

Study reference:	**W. I. L. Research (1997). Twenty-eight day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 3/18/1997** **HERO ID: 787758**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	19. Blinding of Assessors	The study states that investigators were blinded for subjective outcomes in the neurological tests (For FOB parameters "testing was performed by the same technicians without knowledge of the animal group assignment"). No other subjective outcomes were reported in the study.	High	1	1	1
	20. Negative Control Response	The biological responses of the negative control groups were adequate.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	There were no reported differences among the study groups related to confounding variables in test design or procedures and no significant differences in initial body weights.	High	1	2	2

Study reference:	W. I. L. Research (1997). Twenty-eight day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 3/18/1997 **HERO ID: 787758**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	22. Health Outcomes Unrelated to Exposure	Data on attrition and health outcomes unrelated to exposure were reported. The authors report that "animal no. 50292 was replaced by animal no.50289 on study day -1 as animal no. 50292 died shortly after being handled for pretest clinical observations and weighing." The authors also stated that "Several animals weighed less than the protocol-specified minimum weight (175 g for males, 125 g for females) at the initiation of dosing. This deviation had no impact on the outcome of the study as all animals were within the protocol-specified age range (4-8 weeks) at the initiation of dosing. "	Medium	2	1	2
Data Presentation and Analysis	23. Statistical Methods	Statistical methods were clearly described and appropriate for the datasets.	High	1	1	1
	24. Reporting of Data	Data for exposure-related findings were presented for all outcomes by exposure group and sex with quantal or continuous presentation and negative findings reported qualitatively or quantitatively.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**39**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.3**	**Overall Score: Nearest *:**	**1.3**
		Overall Quality Level:		**High**		

2.9 Animal toxicity evaluation results of Wang et al 2016for 28 day oral gavage metabolomic study in mice study on nutrition and metabolic/adult exposure body weight, gene expression/omics outcomes

Study reference:	**Wang, D.,Zhang, P.,Wang, X.,Wang, Y.,Zhou, Z.,Zhu, W. (2016). NMR- and LC-MS/MS-based urine metabolomic investigation of the subacute effects of hexabromocyclododecane in mice Environmental Science and Pollution Research, 23(9), 8500-8507** **HERO ID: 3350496**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified as technical HBCD with 10% alpha, 10% beta, and 80% gamma stereoisomers.	High	1	2	2
	2. Test Substance Source	Test substance obtained from manufacturer but without certification or analytical verification of identity.	Medium	2	1	2
	3. Test Substance Purity	Test substance purity reported as 95%	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Sham-treated controls received vehicle	High	1	2	2
	5. Positive Controls	Positive controls not typical for study type	Not Rated	NR	NR	NR
	6. Randomized Allocation	Study reports random allocation to groups	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Test substance preparation was reported but storage was not reported	Medium	2	1	2
	8. Consistency of Exposure Administration	Time of day of gavage administration was not reported.	Medium	2	1	2
	9. Reporting of Doses/Concentrations	Details of exposure administration were reported and exposures were administered consistently across study groups in a scientifically sound manner	High	1	2	2
	10. Exposure Frequency and Duration	Doses administered daily for 28 days	High	1	1	1

Study reference:	**Wang, D.,Zhang, P.,Wang, X.,Wang, Y.,Zhou, Z.,Zhu, W. (2016). NMR- and LC-MS/MS-based urine metabolomic investigation of the subacute effects of hexabromocyclododecane in mice Environmental Science and Pollution Research, 23(9), 8500-8507** **HERO ID: 3350496**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Number of Exposure Groups and Dose Spacing	2 nonzero doses were administered ranging 5-fold. Doses were selected based on reported range of toxic doses	Medium	2	1	2
	12. Exposure Route and Method	oral gavage exposure with appropriate vehicle reported	High	1	1	1
Test Organism	13. Test Animal Characteristics	Test animal species, strain, sex, age, and body weight were reported. Females were chosen because they were reportedly more sensitive.	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Relative humidity and diet were not reported. All other husbandry conditions were reported and adequate.	Medium	2	1	2
	15. Number per Group	5 animals/dose tested.	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	Body weight, organ weight and both targeted and untargeted metabolomics were evaluated. BW was measured weekly, but metabolomics only performed once on 24 hour urine samples collected after last dose.	Medium	2	2	4
	17. Consistency of Outcome Assessment	No inconsistencies in outcome assessment were noted	High	1	1	1
	18. Sampling Adequacy	Body weights and metabolomics assessed for individual animals	High	1	1	1
	19. Blinding of Assessors	no subjective outcomes	Not Rated	NR	NR	NR
	20. Negative Control Response	Control responses were reported and appeared to be appropriate	High	1	1	1

Study reference:	**Wang, D.,Zhang, P.,Wang, X.,Wang, Y.,Zhou, Z.,Zhu, W. (2016). NMR- and LC-MS/MS-based urine metabolomic investigation of the subacute effects of hexabromocyclododecane in mice Environmental Science and Pollution Research, 23(9), 8500-8507** **HERO ID: 3350496**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	Food and water intake were not reported.	Medium	2	2	4
	22. Health Outcomes Unrelated to Exposure	One control mouse died during the study.	Medium	2	1	2
Data Presentation and Analysis	23. Statistical Methods	Statistical analysis methods reported and appropriate.	High	1	1	1
	24. Reporting of Data	Body weights reported graphically without measure of variability in supplemental material.	Medium	2	2	4
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**42**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**NR**	**Overall Score: Nearest *:**	**NR**
		Overall Quality Level:		**Medium**		
Study Quality Comment:	**The reviewer downgraded this study's overall quality rating. They noted: Although body weight and organ weights were measured, only average body weight was provided in the supplemental material. The author reports that organ weight data was not shown but did not have any changes. This study mainly focuses on metabolomics using urine samples and analyzing amino acids. Even though it is a 28-day study, no useful information is provided in terms of outcomes for toxicological endpoint. It possibly can be used as a mechanistic supporting study for understanding the metabolic pathway. Note: The original calculated score for this study was 1.4. This value is not presented above because the final rating was changed based on professional judgement.**					

2.10 Animal toxicity evaluation results of Watanabe et al 2010 for 28 day feeding study in mice - mechanistic study, animals also infected with rsv study on nutrition and metabolic/adult exposure body weight, hematological and immune outcomes

Study reference:	**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 International Immunopharmacology, 10(4), 393-397** **HERO ID: 1927692**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Substance reported as HBCD, no CAS # was provided	High	1	2	2
	2. Test Substance Source	Purchased from a commercial source	High	1	1	1
	3. Test Substance Purity	Purity was not reported; no validation was done to assess purity	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	The study indicates there was a control, it is presumed that this was the powdered diet alone. It does not appear as though a vehicle was used?	Medium	2	2	4
	5. Positive Controls	Positive control not necessary	Not Rated	NR	NR	NR
	6. Randomized Allocation	Randomization was not reported	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	Preparation nor storage was reported. Study authors only indicate that HBCD was mixed into a powder diet.	Low	3	1	3
	8. Consistency of Exposure Administration	Control and treated Animals were fed ad libitum	High	1	1	1
	9. Reporting of Doses/Concentrations	Reported as 1% in diet., body weights and food consumption were provided,	High	1	2	2
	10. Exposure Frequency and Duration	Daily for 28 days	High	1	1	1

Study reference:	**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 International Immunopharmacology, 10(4), 393-397** **HERO ID: 1927692**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Number of Exposure Groups and Dose Spacing	Single exposure and control; There was no explanation or justification of chosen dose; not useful for dose-response analysis, but single dose may be appropriate for the endpoints evaluated. There were no responses, so it is unclear whether the dose used was appropriate or not.	Medium	2	1	2
	12. Exposure Route and Method	Standard exposure route and method	High	1	1	1
Test Organism	13. Test Animal Characteristics	Test animals were acceptable	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Animal husbandry was not reported	Low	3	1	3
	15. Number per Group	Study reports use of 6-7 mice/ group; OECD guidelines for 28-day repeated dose study recommends 10 animals/group (5/sex)	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	CK: The outcome assessment methodology addressed the intended outcomes	High	1	2	2
	17. Consistency of Outcome Assessment	Methods were acceptable for what they were looking at.	High	1	1	1
	18. Sampling Adequacy	Sampling was done on all of the mice/group	High	1	1	1
	19. Blinding of Assessors	Histology was not done on HBCD treated animals; there were no other subjective outcomes	Not Rated	NR	NR	NR
	20. Negative Control Response	Control responses were as expected	High	1	1	1

Study reference:	**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 International Immunopharmacology, 10(4), 393-397** **HERO ID: 1927692**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	There were no apparently confounding factors that would influence the outcomes	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	There were no unrelated health outcomes	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	Statistical method was appropriate for outcome	High	1	1	1
	24. Reporting of Data	Reporting of data was acceptable	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**41**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**NR**	**Overall Score: Nearest *:**	**NR**
		Overall Quality Level:		**Medium**		
Study Quality Comment:	**The reviewer downgraded this study's overall quality rating. They noted: Some study details regarding preparation of diets, and validation of dosing were omitted. Since there was no justification of dose, it is unknown whether the dose used was appropriate to elicit an effect. The limited endpoints evaluated do not greatly inform mechanism of the potential effects of HBCD on immunity. Note: The original calculated score for this study was 1.4. This value is not presented above because the final rating was changed based on professional judgement.**					

3 Subchronic Toxicity Studies

3.1 Animal toxicity evaluation results of ACC et al 2002 for 90-day gavage-systemic with sperm evaluations and neurobehavior, same as (2990994) study on reproductive, hematological, neurological/behavior, renal, hepatic, clinical chemistry/biochemical , body weight, ocular and sensory, thyroid outcomes

Study reference:	ACC (2002). A 90-Day Oral (Gavage) Toxicity Study of HBCD in Rats **HERO ID: 4269953**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Identified by name, CARSN, structure, molecular formula, and isomer distribution (pp. 1235-1236)	High	1	2	2
	2. Test Substance Source	Source and analytical verification were included in the study report.	High	1	1	1
	3. Test Substance Purity	The test substance composition was such that any observed effects were highly likely to be due to the test substance. Although the test chemical was analyzed to determine the isomer composition analysis does not appear to address the purity of the chemical.	Medium	2	1	2
Test Design	4. Negative and Vehicle Controls	Concurrent vehicle control groups were included in the main and satellite studies.	High	1	2	2
	5. Positive Controls	This metric not applicable.	Not Rated	NR	NR	NR
	6. Randomized Allocation	Animals were allocated by a computerized randomization procedure based on body weight stratification in a block design.	Medium	2	1	2
Exposure Characterization	7. Preparation and Storage of Test Substance	Preparation and storage conditions were reported and appropriate based on stability and homogeneity testing (pp. 1242-1268).	High	1	1	1

Study reference:	ACC (2002). A 90-Day Oral (Gavage) Toxicity Study of HBCD in Rats **HERO ID: 4269953**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	8. Consistency of Exposure Administration	Details were reported and administered consistently across groups. Dosing volume was appropriate. A dosing error was reported (pp. 65) but this is unlikely to have substantial impact on results.	Medium	2	1	2
	9. Reporting of Doses/Concentrations	Doses reported without ambiguity.	High	1	2	2
	10. Exposure Frequency and Duration	Duration of study and frequency of dosing were reported and appropriate for this study	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	The selected doses were not justified by study authors, but the doses were adequate to show results relevant to the outcomes of interest.	Medium	2	1	2
	12. Exposure Route and Method	Exposure route and method were suitable.	High	1	1	1
Test Organism	13. Test Animal Characteristics	The test animal species, strain, sex, health status, age, and starting body weight were reported. Animals obtained from commercial supplier (Charles River).	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Temperature, relative humidity, light/day cycle were reported.	High	1	1	1
	15. Number per Group	In general, the number of animals assigned per group was appropriate for the study type and outcome analysis. Group sizes conformed to OECD 408.	High	1	1	1

Study reference:	ACC (2002). A 90-Day Oral (Gavage) Toxicity Study of HBCD in Rats **HERO ID: 4269953**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	16. Outcome Assessment Methodology	In general, outcome assessment methodology was described in detail and sensitive for outcomes of interest. Serious concerns were identified for serum hormone data. Specifically, the confidence rating for TSH data is low because of a high incidence of samples in the control group below the limit of detection, indicating insensitivity of the method. In one instance data were reported for a single control animal (278-281; 916-939)	High	1	2	2
	17. Consistency of Outcome Assessment	Details of the protocols used for outcome assessment were reported ad outcomes were assessed consistently across study groups.	High	1	1	1
	18. Sampling Adequacy	Sampling details were well described and adequate.	High	1	1	1
	19. Blinding of Assessors	Two subjective outcomes were evaluated: functional observational battery and histopathology. Functional Observational Battery : High - the study report indicates that assessors were blinded to treatment group during observations. Histopathology: Medium - Blinding was not reported in the study and no indication that tissues were subjected to a secondary independent evaluation.	High	1	1	1

Study reference:	ACC (2002). A 90-Day Oral (Gavage) Toxicity Study of HBCD in Rats **HERO ID: 4269953**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	20. Negative Control Response	In general, biological response of negative controls was adequate. Serious concerns were identified for the serum hormone data. Specifically, the confidence rating for TSH data is low because of a high variability in the biological responses between control replicates such that, in some cases, the SD > mean and there were as much as two orders of magnitude difference across individual controls (pp. 278-281; 916-939).	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	No reported differences among the groups were observed.	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	There were no health outcomes unrelated to exposure that would influence outcome assessment.	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	Statistical methods were clearly described and appropriate.	High	1	1	1
	24. Reporting of Data	Data were reported in tables and in the text for all outcomes.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**34**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.1333**	**Overall Score: Nearest *:**	**1.1**
		Overall Quality Level:		**High**		

3.2 Animal toxicity evaluation results of BASF et al 1990 for 28-day and 90-day dietary studies study on reproductive, hematological and immune, neurological, renal, hepatic, endocrine, gastrointestinal, respiratory, thyroid outcomes

Study reference:	BASF (1990). Hexabromocyclododecane 28-day feeding trials with rats with test data and cover letter, 900000274, #86-900000274 **HERO ID: 787638**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Identified by trade name and isomer designation.	High	1	2	2
	2. Test Substance Source	Source and lot no. were not reported. Manufacturer was assumed to be BASF.	Medium	2	1	2
	3. Test Substance Purity	Purity was not reported.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	A negative dietary control group was used.	High	1	2	2
	5. Positive Controls	Positive controls are not necessary for a 28-day study.	Not Rated	NR	NR	NR
	6. Randomized Allocation	The study did not report how animals were allocated to study groups.	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	Analysis showed that concentrations remained stable over the week.	High	1	1	1
	8. Consistency of Exposure Administration	Details of exposure administration were reported.	High	1	1	1
	9. Reporting of Doses/Concentrations	Dietary concentrations were not measured analytically, but bw and food consumption were reported for each group.	Medium	2	2	4
	10. Exposure Frequency and Duration	Diet was administered over 13 weeks (daily was assumed).	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	4 treatment groups plus control; dose response relationships were apparent.	High	1	1	1
	12. Exposure Route and Method	The route and method of exposure were reported and were suited to the test substance.	High	1	1	1

Study reference:	**BASF (1990). Hexabromocyclododecane 28-day feeding trials with rats with test data and cover letter, 900000274, #86-900000274** **HERO ID: 787638**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Organism	13. Test Animal Characteristics	Species, strain and starting bw were reported. Not a commercial source, but a laboratory maintained colony.	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Husbandry conditions were not reported.	Low	3	1	3
	15. Number per Group	10/sex/group	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	The outcome assessment methodology was reported.	High	1	2	2
	17. Consistency of Outcome Assessment	See footnote at end of page.[1]	High	1	1	1
	18. Sampling Adequacy	Data tables are difficult to read, but sampling appears adequate.	Medium	2	1	2
	19. Blinding of Assessors	Blinding was not reported; however, outcomes were objective.	Medium	2	1	2
	20. Negative Control Response	Data tables are difficult to read; however, several lesions are noted for controls.	Low	3	1	3
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	The study reported (in the text) minor differences among the study groups (<20% difference from control) with respect to initial body weight, drinking water and/or food consumption. But the information in the tables is difficult to read.	Medium	2	2	4

[1] Metrics that received a "High" rating met the criteria as discussed in the Applications of Systematic Review for TSCA Risk Evaluation.

Study reference:	BASF (1990). Hexabromocyclododecane 28-day feeding trials with rats with test data and cover letter, 900000274, #86-900000274 **HERO ID: 787638**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	22. Health Outcomes Unrelated to Exposure	A large proportion of rats showed signs of respiratory inflammation (47% of controls, 26% of treated rats) which would not be expected from a feeding trial.	Unacceptable	4	1	4
Data Presentation and Analysis	23. Statistical Methods	Statistical analysis was not described clearly, and this deficiency is likely to have a substantial impact on results.	Low	3	1	3
	24. Reporting of Data	Data tables are provided for all outcomes by exposure group and sex; however, data are in German and mostly illegible.	Low	3	2	6
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**54**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.8000**	**Overall Score (Rounded):**	**1.8[1]**
		Overall Quality Level:		**Unacceptable[1]**		
Comment:	**Footnote:** **[1] Consistent with our *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, seven of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.**					

3.3 Animal toxicity evaluation results of van et al 2009 for 1-generation reproduction study, oral dietary study on endocrine; reproductive; hematological and immune; thyroid; growth (early life) and development; musculoskeletal/motor function; clinical chemistry/biochemical; nutrition and metabolic/adult exposure body weight; hepatic outcomes

Study reference:	van der Ven, L. T. M.,van de Kuil, T.,Leonards, P. E. G.,Slob, W.,Lilienthal, H.,Litens, S.,Herlin, M.,Hakansson, H.,Cantón, R. F.,van den Berg, M.,Visser, T. J.,van Loveren, H.,Vos, J. G.,Piersma, A. H. (2009). Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats Toxicology Letters, 185(1), 51-62 **HERO ID: 589273**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	The test substance was identified definitively as HBCD a mixture of three diastereoisomers, H alpha-, beta-, and gamma- HBCD and their respective proportion in the used batch was 10.3–8.7–81.0%.	High	1	2	2
	2. Test Substance Source	The test substance manufacturer and source were reported; however, the batch/lot number was not specified.	Medium	2	1	2
	3. Test Substance Purity	The test substance was said to be technical grade (technical mixture containing traces of tetra- and pentabromocyclododecane) it was noted; the test substance composition is such that any observed effects are likely due to the nominal test substance.	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Study authors reported using an appropriate concurrent negative control group. An additional group was included to monitor effects of the carrier oil contents in the feed.	High	1	2	2
	5. Positive Controls	This metric is not rated/applicable for this study type	Not Rated	NR	NR	NR

Study reference:	van der Ven, L. T. M.,van de Kuil, T.,Leonards, P. E. G.,Slob, W.,Lilienthal, H.,Litens, S.,Herlin, M.,Hakansson, H.,Cantón, R. F.,van den Berg, M.,Visser, T. J.,van Loveren, H.,Vos, J. G.,Piersma, A. H. (2009). Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats Toxicology Letters, 185(1), 51-62 **HERO ID: 589273**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	6. Randomized Allocation	The study noted that the protocol was based on OECD415 (one-generation reproduction toxicity study) guideline and that the animals were distributed among a larger number of dose groups than advised in guideline. The study did not explicitly report how animals were allocated to study groups. It is unclear if this would have a substantial impact on results.	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	Test substance preparation was reported, but with limitations in reporting. HBCD was reported to be mixed with corn-based oil and pelleted for feed. It is not reported how often feed was mixed or how it was stored. This omission is unlikely to have a substantial impact on results.	Medium	2	1	2
	8. Consistency of Exposure Administration	Details of exposure administration were reported and administration was consistent between across study groups.	High	1	1	1
	9. Reporting of Doses/Concentrations	The targeted dietary exposure was reported to be 0–0.1–0.3–1–3–10–30–100 mg/kg bodyweight/day.	High	1	2	2
	10. Exposure Frequency and Duration	Exposure frequency (ad libitum) and duration of exposure were reported and appropriate.	High	1	1	1

Study reference:	van der Ven, L. T. M.,van de Kuil, T.,Leonards, P. E. G.,Slob, W.,Lilienthal, H.,Litens, S.,Herlin, M.,Hakansson, H.,Cantón, R. F.,van den Berg, M.,Visser, T. J.,van Loveren, H.,Vos, J. G.,Piersma, A. H. (2009). Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats Toxicology Letters, 185(1), 51-62 **HERO ID: 589273**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Number of Exposure Groups and Dose Spacing	The number of exposure groups and spacing was reported and was justified based on a preceding subacute repeated oral dose study.	High	1	1	1
	12. Exposure Route and Method	The route (oral, dietary) was reported and suited to the test substance.	High	1	1	1
Test Organism	13. Test Animal Characteristics	The test animal species, strain, sex, and age were reported. It was noted that the animals were of weighed and that animals were inspected daily for general condition and clinical abnormalities. The animals were obtained from a commercial breeding facility.	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Animal husbandry conditions were reported and included temperature, humidity, and light-dark cycle. Husbandry conditions were adequate and the same for all animals.	High	1	1	1
	15. Number per Group	The number of animals per group was reported and appropriate for the study type and outcome analysis.	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	The outcome assessment methodology reported and sensitive to the intended outcomes of interest.	High	1	2	2
	17. Consistency of Outcome Assessment	Details of the outcome assessment methodology were reported and consistent across study groups for the outcomes of interest.	High	1	1	1

Study reference:	**van der Ven, L. T. M.,van de Kuil, T.,Leonards, P. E. G.,Slob, W.,Lilienthal, H.,Litens, S.,Herlin, M.,Hakansson, H.,Cantón, R. F.,van den Berg, M.,Visser, T. J.,van Loveren, H.,Vos, J. G.,Piersma, A. H. (2009). Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats Toxicology Letters, 185(1), 51-62** **HERO ID: 589273**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	18. Sampling Adequacy	Details regarding the sampling for the outcomes of interest were reported and adequate for assessment.	High	1	1	1
	19. Blinding of Assessors	This metric is not rated when outcomes are not subjective or for initial histopathology review.	Not Rated	NR	NR	NR
	20. Negative Control Response	The biological response of the negative control group was adequate. As shown in Supplemental tables 1-16 (ID2919529)	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	There were no reported differences among the study groups that could influence the outcome assessment.	Medium	2	2	4
	22. Health Outcomes Unrelated to Exposure	Data on attrition or health outcomes not related to exposure were not reported. The carrier oil control group experienced increased mortality of F1 pups during lactation and several other health outcomes. While not related to HBDC exposure, these effects were influenced by the carrier oil in the feed.	Medium	2	1	2
Data Presentation and Analysis	23. Statistical Methods	Statistical analysis was shown for all datasets as evaluated for Supplemental tables 1-16 (ID2919529). BMD methodology was clearly described and appropriate.	High	1	1	1

Study reference:	**van der Ven, L. T. M.,van de Kuil, T.,Leonards, P. E. G.,Slob, W.,Lilienthal, H.,Litens, S.,Herlin, M.,Hakansson, H.,Cantón, R. F.,van den Berg, M.,Visser, T. J.,van Loveren, H.,Vos, J. G.,Piersma, A. H. (2009). Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats Toxicology Letters, 185(1), 51-62** **HERO ID: 589273**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	24. Reporting of Data	Data for exposure-related findings were presented for all outcomes by exposure group and sex - as evaluated for Supplemental tables 1-16 (ID2919529).	High	1	2	2
High: >=1 and <1.7 **Medium: >=1.7 and <2.3** **Low: >=2.3 and <=3**		**Sum of scores:**			**29**	**36**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.2414**	**Overall Score: Nearest *:**	**1.2**
		Overall Quality Level:		**High**		

3.4 Animal toxicity evaluation results of W. I. L. Research 2001 for 90-day gavage study on reproductive, hematological and immune, neurological/behavior, renal, hepatic, ocular and sensory, cardiovascular, clinical chemistry/biochemical, endocrine, gastrointestinal, body weight, respiratory, thyroid outcomes

Study reference:	**W. I. L. Research (2001). 90-Day oral (gavage) toxicity study of HBCD in rats** **HERO ID: 787787**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Identified by name.	High	1	2	2
	2. Test Substance Source	Manufacturer, lot no. and composite sample nos.	High	1	1	1
	3. Test Substance Purity	Composite made from commercial HBCD products. CK: HBCD, Alpha; HBCD, Beta; HBCD, Gamma; CAS number 3194-55-6. The standards had reported purities of 99.4%,100% and 98.7%. respectively,	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Vehicle (corn oil) controls were used.	High	1	2	2
	5. Positive Controls	Positive controls are not used for 90-day studies.	Not Rated	NR	NR	NR
	6. Randomized Allocation	Computerized randomization.	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Stirred until uniform and continuously throughout used. Dosing formulations were prepared weekly.	High	1	1	1
	8. Consistency of Exposure Administration	See footnote at end of page.[1]	High	1	1	1

[1] Metrics that received a "High" rating met the criteria as discussed in the Applications of Systematic Review for TSCA Risk Evaluation.

	9. Reporting of Doses/Concentrations	Doses reported as mg/kg/day, based on most recent bw measurement.	High	1	2	2
	10. Exposure Frequency and Duration	90 consecutive days.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	3 treatment groups plus control; not justified by authors, but did produce a range of response (i.e., thyroid).	High	1	1	1
	12. Exposure Route and Method	CK: Followed OECD Guidelines OECD Guideline 408 and OPPTS 870.3 100	High	1	1	1
Test Organism	13. Test Animal Characteristics	Species, strain, sex, age, and starting body weight were reported (commercial source).	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Husbandry conditions were reported and appropriate.	High	1	1	1
	15. Number per Group	15/sex/group	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Thorough outcome assessments.	High	1	2	2
	17. Consistency of Outcome Assessment	See footnote at end of page.[1]	High	1	1	1
	18. Sampling Adequacy	See footnote at end of page.[1]	High	1	1	1
	19. Blinding of Assessors	FOB testing was performed without knowledge of the animal groups assignment. Other outcomes were objective. CK: Functional Observational Battery (FOB) evaluations	High	1	1	1
	20. Negative Control Response	Low incidence of histopath. lesions.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	See footnote at end of page.[1]	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	See footnote at end of page.[1]	High	1	1	1

Data Presentation and Analysis	23. Statistical Methods	CK: Well described	High	1	1	1
	24. Reporting of Data	Summary and individual animals tables were included.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**30**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1**	**Overall Score: Nearest *:**	**1**
		Overall Quality Level:		**High**		

[1] Metrics that received a "High" rating met the criteria as discussed in the Applications of Systematic Review for TSCA Risk Evaluation.

3.5 Animal toxicity evaluation results of Ema et al 2008 study on reproductive, growth (early life) and development, hepatic, neurological/behavior, thyroid outcomes

Study reference:	**Ema, M.,Fujii, S.,Hirata-Koizumi, M.,Matsumoto, M. (2008). Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats Reproductive Toxicology, 25(3), 335-351** **HERO ID: 787657**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	The CASRN, purity, mixture components, and ratios were explicitly specified.	High	1	2	2
	2. Test Substance Source	The manufacturer was specified; test substance number was reported. It was indicated that the purity and stability of the test chemical were verified using liquid chromatography.	High	1	1	1
	3. Test Substance Purity	The test substance was 99.7% pure; therefore, effects in the study were highly likely to be due to the test substance itself (rather than any unspecified impurities).	High	1	1	1
Test Design	4. Negative and Vehicle Controls	An appropriate concurrent control group was used (all of the conditions the same except exposure).	High	1	2	2
	5. Positive Controls	Positive control not indicated by study type.	Not Rated	NR	NR	NR
	6. Randomized Allocation	The study indicates that rats were randomly assigned into study groups.	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	It was indicated that the test substance was stored in a sealed container under cool and dark conditions. The test substance was well-mixed in the diet (homogeneous and stable for at least 21 days).	High	1	1	1

Study reference:	**Ema, M.,Fujii, S.,Hirata-Koizumi, M.,Matsumoto, M. (2008). Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats Reproductive Toxicology, 25(3), 335-351** **HERO ID: 787657**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	8. Consistency of Exposure Administration	Analysis of the diet indicated that the test substance was administered at the desired feed concentrations throughout the study. Animals were fed ad libitum.	High	1	1	1
	9. Reporting of Doses/Concentrations	Food consumption data were recorded (provided in the supplemental data). Mean daily intakes of the test substance for various generations and life stages (i.e. F0 and F1 males and females during pre-mating, mating, gestation, lactation, and for the whole period of administration) were reported without ambiguity.	High	1	2	2
	10. Exposure Frequency and Duration	The exposure frequency and duration were appropriate for the study type (and consistent with OECD guidelines). Mating was 3 weeks (rather than 2 weeks outlined by guideline).	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	Three dose groups and a concurrent control group were used. Dosage levels were based on the results of a 90-day repeated-dose toxicity study.	High	1	1	1
	12. Exposure Route and Method	The test substance was administered in the diet (oral route is recommended by guideline).	High	1	1	1

Study reference:	**Ema, M.,Fujii, S.,Hirata-Koizumi, M.,Matsumoto, M. (2008). Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats Reproductive Toxicology, 25(3), 335-351** **HERO ID: 787657**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Organism	13. Test Animal Characteristics	The animal species, strain, sex, health, age, and starting body weights were reported. Animals were purchased from a commercial laboratory. Crl:CD(SD) rats were used because they are the most commonly used in reproductive and developmental toxicity studies; historical control data are available. The rat is the preferred species for testing (according to guideline).	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Animals were housed under the same conditions (at the temperature and humidity recommended by guideline). Animals were housed individually except during acclimation, mating, and nursing periods.	High	1	1	1
	15. Number per Group	No less than 20 pregnant females per group is preferred (but not always possible). The study utilized 24 rats/sex/group. Although the number of pregnant animals was only 19 for high-dose F0 females, the number of pregnant females was adequate for meaningful analyses of the desired outcomes.	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	The outcome assessment methodology addressed the intended outcomes (mirrored guideline recommendations for a two-generation reproductive toxicity assay).	High	1	2	2

Study reference:	Ema, M.,Fujii, S.,Hirata-Koizumi, M.,Matsumoto, M. (2008). Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats Reproductive Toxicology, 25(3), 335-351 **HERO ID: 787657**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	17. Consistency of Outcome Assessment	The outcomes were measured consistently across study groups.	High	1	1	1
	18. Sampling Adequacy	Reporting details were provided; litter data were recorded. Sampling was adequate for the outcomes of interest.	High	1	1	1
	19. Blinding of Assessors	Although the study does not indicate that investigators were blinded to treatment group, the study cited various quality control methods that were followed.	High	1	1	1
	20. Negative Control Response	The response of the negative controls was reported and were adequate (e.g. there were no histological findings in the thyroid of control rats).	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	There were no differences in initial body weights or intake that could influence the outcome assessment.	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	Details regarding animal outcomes unrelated to exposure (i.e. accidental injury in the home cage) were reported, but these differences would not influence the outcome assessment.	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	Statistical methods were clearly described.	High	1	1	1

Study reference:	Ema, M.,Fujii, S.,Hirata-Koizumi, M.,Matsumoto, M. (2008). Two-generation reproductive toxicity study of the flame retardant hexabromocyclododecane in rats Reproductive Toxicology, 25(3), 335-351 **HERO ID: 787657**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	24. Reporting of Data	Data were provided for all exposure-related findings by dose group. The cutoff value for decreased thyroid follicle size was not reported, but this is not likely to affect the outcome of the study. Additional data are provided in the supplemental document (for example, date for primordial follicles are presented graphically in the primary report; quantitative data are available in the supplemental document).	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**30**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1**	**Overall Score: Nearest *:**	**1**
		Overall Quality Level:		**High**		

3.6 Animal toxicity evaluation results of Lilienthal et al 2009 (787693) for 1-generation reproductive study, dietary exposure study on neurological/behavior outcomes

Study reference:	**Lilienthal, H.,van der Ven, L. T.,Piersma, A. H.,Vos, J. G. (2009). 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 Toxicology Letters, 185(1), 63-72** **HERO ID: 787693**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Isomer composition of HBCD was reported.	High	1	2	2
	2. Test Substance Source	Supplier was Bromine Science and Environmental Forum. No information on lot or batch and no analytical verification was described.	Medium	2	1	2
	3. Test Substance Purity	HBCD was a technical mixture of three diastereoisomers, alpha, beta, and gamma-HBCD at respective proportions of 10.28%, 8.72%, and 81.02% with traces of tetra- and pentabromocyclododecane.	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Untreated and vehicle controls.	High	1	2	2
	5. Positive Controls	Positive controls were not needed for neurobehavioral studies.	Not Rated	NR	NR	NR
	6. Randomized Allocation	The study did not report how animals were allocated to study groups.	Low	3	1	3
Exposure Characterization	7. Preparation and Storage of Test Substance	Preparation of test diets was described; however, the frequency of preparation and store was not indicated.	Medium	2	1	2
	8. Consistency of Exposure Administration	Details of exposure administration were reported, and exposures were administered consistently across study groups in a scientifically sound manner.	High	1	1	1

Study reference:	**Lilienthal, H.,van der Ven, L. T.,Piersma, A. H.,Vos, J. G. (2009). 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 Toxicology Letters, 185(1), 63-72** **HERO ID: 787693**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	9. Reporting of Doses/Concentrations	Dose in mg/kg/day were calculated by study authors.	High	1	2	2
	10. Exposure Frequency and Duration	Continuous paternal and maternal exposure during premating, mating, gestation, lactation and after weaning in offspring was reported.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	The number of exposure groups and dose/concentration spacing were justified by study authors and considered adequate to address the purpose of the study.	High	1	1	1
	12. Exposure Route and Method	The route and method of exposure were reported and were suited to the test substance.	High	1	1	1
Test Organism	13. Test Animal Characteristics	Species, strain, sex and starting age were provided (commercial source).	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Husbandry conditions were reported and appropriate.	Medium	2	1	2
	15. Number per Group	6/sex/group	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	The outcome assessment methodology addressed or reported the intended outcome(s) of interest and was sensitive for the outcomes(s) of interest.	High	1	2	2
	17. Consistency of Outcome Assessment	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups.	High	1	1	1

Study reference:	Lilienthal, H.,van der Ven, L. T.,Piersma, A. H.,Vos, J. G. (2009). 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 Toxicology Letters, 185(1), 63-72 **HERO ID: 787693**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	18. Sampling Adequacy	Details regarding sampling for the outcome(s) of interest were reported.	High	1	1	1
	19. Blinding of Assessors	The authors report that "personnel conducting the measurements were unaware of the exposure conditions" suggesting the assessors were blinded.	High	1	1	1
	20. Negative Control Response	The biological responses of the negative control group(s) were adequate.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	Initial body weight and food/water intake were not reported.	Low	3	2	6
	22. Health Outcomes Unrelated to Exposure	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.	Low	3	1	3
Data Presentation and Analysis	23. Statistical Methods	Statistics and BMD modeling was reported.	High	1	1	1
	24. Reporting of Data	Test data and BMD results were reported.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**41**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.3667**	**Overall Score: Nearest *:**	**1.4**
		Overall Quality Level:		**High**		

3.7 Animal toxicity evaluation results of Saegusa et al 2009 for 1-generation developmental toxicity (dietary exposure) study on reproductive, growth (early life) and development, neurological, hepatic, endocrine, thyroid, nutrition and metabolic/adult exposure body weight outcomes

Study reference:	**Saegusa, Y.,Fujimoto, H.,Woo, G. H.,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 Reproductive Toxicology, 28(4), 456-467** **HERO ID: 787721**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Identified by chemical name and CASRN.	High	1	2	2
	2. Test Substance Source	Manufacturer and lot no. were reported..	High	1	1	1
	3. Test Substance Purity	>95%	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Concurrent negative control.	High	1	2	2
	5. Positive Controls	Positive control not needed developmental studies.	Not Rated	NR	NR	NR
	6. Randomized Allocation	Randomized allocation.	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Test substance preparation and storage were not described.	Low	3	1	3
	8. Consistency of Exposure Administration	Details of exposure administration were reported.	High	1	1	1
	9. Reporting of Doses/Concentrations	Doses were reported as mg/kg-day (mean +/- SD) for 3 time periods (GD 10-20, PND 1-9 and PND 10-20)	High	1	2	2
	10. Exposure Frequency and Duration	Daily exposure during critical developmental periods.	High	1	1	1
	11. Number of Exposure Groups and Dose Spacing	Range-finding study was used to set doses: 3 treatment groups plus controls.	High	1	1	1

Study reference:	**Saegusa, Y.,Fujimoto, H.,Woo, G. H.,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 Reproductive Toxicology, 28(4), 456-467** **HERO ID: 787721**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	12. Exposure Route and Method	The route and method of exposure were reported and were suited to the test substance.	High	1	1	1
Test Organism	13. Test Animal Characteristics	Test animals were obtained from a commercial source. Species, strain, and pregnancy status were reported.	High	1	2	2
	14. Adequacy and Consistency of Animal Husbandry Conditions	Husbandry conditions were reported and appropriate.	High	1	1	1
	15. Number per Group	The number of animals per study group was reported, appropriate for the study type and outcome analysis, and consistent with studies of the same or similar type (10/group).	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Thorough outcome examinations pubertal and adult necropsies).	High	1	2	2
	17. Consistency of Outcome Assessment	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.	High	1	1	1
	18. Sampling Adequacy	Details regarding sampling for the outcome(s) of interest were reported and the study used adequate sampling for the outcome(s) of interest (e.g., litter data provided for developmental studies; endpoints were evaluated in an adequate number of animals in each group).	High	1	1	1

Study reference:	**Saegusa, Y.,Fujimoto, H.,Woo, G. H.,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 Reproductive Toxicology, 28(4), 456-467** **HERO ID: 787721**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	19. Blinding of Assessors	Blinding was not reported, but outcomes were objective.	Medium	2	1	2
	20. Negative Control Response	No histopathology lesion in controls.	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	No differences among groups in food consumption and body weight.	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group	Low	3	1	3
Data Presentation and Analysis	23. Statistical Methods	Statistical methods were clearly described and appropriate for dataset(s).	High	1	1	1
	24. Reporting of Data	HBCD caused a dose-dependent decrease in Cingulate deep cortex CNPase (+) cell count, which was significantly lower at the highest dose exposed.	Medium	2	2	4
High: >=1 and <1.7 **Medium: >=1.7 and <2.3** **Low: >=2.3 and <=3**		**Sum of scores:**			**30**	**37**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.2333**	**Overall Score: Nearest *:**	**1.2**
		Overall Quality Level:		**High**		

3.8 Animal toxicity evaluation results of Yanagisawa et al 2014 for 14-week study (animals dosed by gavage 1x per week) study on hepatic, body weight, nutrition and metabolic/adult exposure body weight outcomes

Study reference:	**Yanagisawa, R.,Koike, E.,Win-Shwe, T. T.,Yamamoto, M.,Takano, H. (2014). Impaired lipid and glucose homeostasis in hexabromocyclododecane-exposed mice fed a high-fat diet Environmental Health Perspectives, 122(3), 277-283** **HERO ID: 2343717**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance described as HBCD, study did not indicate whether the test substance was composed of different isomers (as other studies have).	Medium	2	2	4
	2. Test Substance Source	Sigma Aldrich - no catalog #	High	1	1	1
	3. Test Substance Purity	Purity was not reported, however, products purchased from Sigma for experimental use are generally >95% pure.	Medium	2	1	2
Test Design	4. Negative and Vehicle Controls	an appropriate vehicle control was used	High	1	2	2
	5. Positive Controls	Positive control was not necessary	Not Rated	NR	NR	NR
	6. Randomized Allocation	Mice were randomly allocated. There were no differences in initial BWs	High	1	1	1
Exposure Characterization	7. Preparation and Storage of Test Substance	Preparation of the test substance was described, but the frequency of preparation and storage were not reported.	Medium	2	1	2
	8. Consistency of Exposure Administration	All groups appeared to be treated consistently	High	1	1	1
	9. Reporting of Doses/Concentrations	Dosing was clearly reported, although reported as mg/kg/week CK: Dosing was reported as µg/kg BW/week, not as mg/kg/week	High	1	2	2

Study reference:	**Yanagisawa, R.,Koike, E.,Win-Shwe, T. T.,Yamamoto, M.,Takano, H. (2014). Impaired lipid and glucose homeostasis in hexabromocyclododecane-exposed mice fed a high-fat diet Environmental Health Perspectives, 122(3), 277-283** **HERO ID: 2343717**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	10. Exposure Frequency and Duration	Animals were only given the test substance 1x/week via oral gavage. This is not a standard frequency of administration, and there is no discussion in the text indicating reasoning for the chosen dosing frequency. It is an unusual frequency to evaluate the toxicological effects of the test substance on mice fed different diets.	Unacceptable	4	1	4
	11. Number of Exposure Groups and Dose Spacing	Three exposure groups and a control. Justification for exposure levels was provided.	High	1	1	1
	12. Exposure Route and Method	Method of gavage is acceptable, although it is unclear in this case, why a spiked dietary administration wasn't used instead.	High	1	1	1
Test Organism	13. Test Animal Characteristics	Animals, and animal characteristics were all reported, however, only males were used, for a ~90-day repeated dose study; OECD guideline recommends testing on both sexes	Medium	2	2	4
	14. Adequacy and Consistency of Animal Husbandry Conditions	Animal husbandry conditions were appropriate	High	1	1	1
	15. Number per Group	Only 5-6 animals/group; OECD guideline for 90-day repeated dose study recommends a minimum of 8 animals/group (4 males and 4 females)	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	Methods used to assess outcomes were appropriate	High	1	2	2

Study reference:	**Yanagisawa, R.,Koike, E.,Win-Shwe, T. T.,Yamamoto, M.,Takano, H. (2014). Impaired lipid and glucose homeostasis in hexabromocyclododecane-exposed mice fed a high-fat diet Environmental Health Perspectives, 122(3), 277-283** **HERO ID: 2343717**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	17. Consistency of Outcome Assessment	There was consistency across the groups that were tested	High	1	1	1
	18. Sampling Adequacy	A number of endpoints were only done using controls and high-dose groups, even though significant changes were supposedly observed in the medium-dose group for other endpoints. This precludes the ability to evaluate dose-response for these endpoints	Medium	2	1	2
	19. Blinding of Assessors	Study indicates histology was done in a blinded fashion.	High	1	1	1
	20. Negative Control Response	No unexpected negative control responses were reported	High	1	1	1
Confounding / Variable Control	21. Confounding Variables in Test Design and Procedures	No confounding variables were identified.	High	1	2	2
	22. Health Outcomes Unrelated to Exposure	No unusual health outcomes un-related to the exposure were identified	High	1	1	1
Data Presentation and Analysis	23. Statistical Methods	Statistical analysis was clearly described and appropriate	High	1	1	1
	24. Reporting of Data	Data presentation was adequate; histological data was presented as images only	Medium	2	2	4
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**30**	**43**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.4333**	**Overall Score (Rounded):**	**1.4[1]**
		Overall Quality Level:		**Unacceptable[1]**		

Study reference:	**Yanagisawa, R.,Koike, E.,Win-Shwe, T. T.,Yamamoto, M.,Takano, H. (2014). Impaired lipid and glucose homeostasis in hexabromocyclododecane-exposed mice fed a high-fat diet Environmental Health Perspectives, 122(3), 277-283** **HERO ID: 2343717**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Comment:	**Footnote:** **[1] Consistent with our *Application of Systematic Review in TSCA Risk Evaluations* document, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, one of the metrics was rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.**					

4 *In Vitro* Studies

4.1 In vitro evaluation results of 1990.

Study reference:	(1990). LETTER FROM AMERIBROM INC TO US EPA REGARDING 8D SUBMISSION FOR HEXABROMOCYCLODODECANE WITH ATTACHMENTS HERO ID: 1928284					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by name, chemical formula, and physical chemical properties.	High	1	2	2
	2. Test Substance Source	Source not identified.	Low	3	1	3
	3. Test Substance Purity	Purity not reported.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Negative controls were included.	High	1	2	2
	5. Positive Controls	Positive controls were included.	High	1	2	2
	6. Assay procedures	Assay procedures were described.	High	1	1	1
	7. Standards for tests	Criteria not required.	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Preparation details were described.	High	1	1	1
	9. Consistency of Exposure Administration	Exposures were administered consistently.	High	1	1	1
	10. Reporting of Doses/Concentrations	Concentrations were reported.	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Duration was reported.	High	1	2	2
	12. Exposure Route and Method	The number of groups and spacing were reported with justification.	High	1	1	1
	13. Metabolic Activation	Activation system and mix were described.	High	1	1	1
Test Model	14. Test Model	Test models were well described.	High	1	2	2

Study reference:	(1990). LETTER FROM AMERIBROM INC TO US EPA REGARDING 8D SUBMISSION FOR HEXABROMOCYCLODODECANE WITH ATTACHMENTS **HERO ID: 1928284**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	An overnight culture was used for experiments, but exact number of cells not reported. The number of replicates was reported.	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methodology was described.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate.	High	1	2	2
	19. Blinding of Assessors	Blinding was not required.	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	No confounding variables were reported.	High	1	2	2
	21. Confounding Variables in Outcomes Unrelated to Exposure	No outcomes unrelated to exposure were reported.	High	1	1	1
Data Presentation and Analysis	22. Data Analysis	Statistical methods were described.	High	1	1	1
	23. Data Interpretation	Criteria for positive finding was described.	High	1	2	2
	24. Cytotoxicity Data	A preliminary cytotoxicity assay was conducted.	High	1	1	1
	25. Reporting of Data	Data were reported.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**34**	**39**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.1471**	**Overall Score: Nearest *:**	**1.1**
		Overall Quality Level:		**High**		

4.2 In vitro evaluation results of Almughamsi et al 2016

Study reference:	**Almughamsi, H.,Whalen, M. M. (2016). Hexabromocyclododecane and tetrabromobisphenol A alter secretion of interferon gamma (IFN-?) from human immune cells Archives of Toxicology, 90(7), 1695-1707** **HERO ID: 3350524**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by name.	Medium	2	2	4
	2. Test Substance Source	Source was identified.	Medium	2	1	2
	3. Test Substance Purity	Purity/grade and/or composition were not reported.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Concurrent controls were included.	High	1	2	2
	5. Positive Controls	Positive controls not required.	Not Rated	NR	NR	NR
	6. Assay procedures	Assay procedures were reported.	High	1	1	1
	7. Standards for tests	No standards were required for the assays.	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Limited preparation details were provided and not storage or stability data were reported.	Medium	2	1	2
	9. Consistency of Exposure Administration	Exposures were administered consistently.	High	1	1	1
	10. Reporting of Doses/Concentrations	Concentrations were reported.	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Durations were reported.	High	1	2	2
	12. Exposure Route and Method	The number of groups and spacing were reported but not justified.	Medium	2	1	2
	13. Metabolic Activation	Not required for the assay.	Not Rated	NR	NR	NR
Test Model	14. Test Model	The test models and sources were identified and appropriate.	High	1	2	2
	15. Number per Group	The number of cells exposure were reported.	High	1	1	1

Study reference:	Almughamsi, H.,Whalen, M. M. (2016). Hexabromocyclododecane and tetrabromobisphenol A alter secretion of interferon gamma (IFN-?) from human immune cells Archives of Toxicology, 90(7), 1695-1707 **HERO ID: 3350524**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methodology was reported.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate.	High	1	2	2
	19. Blinding of Assessors	Blinding not required.	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	No confounding variables in test design were reported.	High	1	2	2
	21. Confounding Variables in Outcomes Unrelated to Exposure	No confounding variables in outcomes unrelated to exposures were reported.	High	1	1	1
Data Presentation and Analysis	22. Data Analysis	Statistical methods were reported and appropriate.	High	1	1	1
	23. Data Interpretation	Metric not required.	Not Rated	NR	NR	NR
	24. Cytotoxicity Data	Cell viability methods were defined and described.	High	1	1	1
	25. Reporting of Data	Data were reported.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**36**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.2414**	**Overall Score: Nearest *:**	**1.2**
		Overall Quality Level:		**High**		

4.3 In vitro evaluation results of An et al 2016

Study reference:	An, J.,Guo, P.,Shang, Y.,Zhong, Y.,Zhang, X.,Yu, Y.,Yu, Z. (2016). The adaptive response; of low concentrations of HBCD in L02 cells and the underlying molecular mechanisms Chemosphere, 145, 68-76 **HERO ID: 3350502**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by name.	Medium	2	2	4
	2. Test Substance Source	Source identified.	Medium	2	1	2
	3. Test Substance Purity	Purity/composition was not reported but was reported to be analytical reagents.	Medium	2	1	2
Test Design	4. Negative and Vehicle Controls	Negative controls were included.	High	1	2	2
	5. Positive Controls	Positive controls not required.	Not Rated	NR	NR	NR
	6. Assay procedures	Assay procedures were described.	High	1	1	1
	7. Standards for tests	No standards were required.	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Preparation, storage, and stability information were not reported.	Low	3	1	3
	9. Consistency of Exposure Administration	Exposures were administered consistently.	High	1	1	1
	10. Reporting of Doses/Concentrations	Concentrations were reported.	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Exposure durations were reported.	High	1	2	2
	12. Exposure Route and Method	The number of exposure groups and spacing were reported and justified.	High	1	1	1
	13. Metabolic Activation	Metabolic activation was not required.	Not Rated	NR	NR	NR
Test Model	14. Test Model	The test model was described with limited details, and the source was not reported.	Medium	2	2	4

Study reference:	An, J.,Guo, P.,Shang, Y.,Zhong, Y.,Zhang, X.,Yu, Y.,Yu, Z. (2016). The adaptive response; of low concentrations of HBCD in L02 cells and the underlying molecular mechanisms Chemosphere, 145, 68-76 **HERO ID: 3350502**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	The number of organisms exposed was not reported for all experiments.	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methodology was reported.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate.	High	1	2	2
	19. Blinding of Assessors	Blinding was not applicable.	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	No confounding variables in test design were reported.	High	1	2	2
	21. Confounding Variables in Outcomes Unrelated to Exposure	No outcomes unrelated to exposure were reported.	High	1	1	1
Data Presentation and Analysis	22. Data Analysis	Statistical methods were reported and appropriate.	High	1	1	1
	23. Data Interpretation	Not required for these assays.	Not Rated	NR	NR	NR
	24. Cytotoxicity Data	Cell viability methods were described.	High	1	1	1
	25. Reporting of Data	Data were reported.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**38**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.3103**	**Overall Score: Nearest *:**	**1.3**
		Overall Quality Level:		**High**		

4.4 In vitro evaluation results of Anisuzzaman et al 2016

Study reference:	**Anisuzzaman, S.,Whalen, M. M. (2016). Tetrabromobisphenol A and hexabromocyclododecane alter secretion of IL-1 from human immune cells Journal of Immunotoxicology, 13(3), 403-416** **HERO ID: 3350463**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by name.	Medium	2	2	4
	2. Test Substance Source	The source was identified.	Medium	2	1	2
	3. Test Substance Purity	Purity was not reported.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Concurrent negative controls were included	High	1	2	2
	5. Positive Controls	Positive controls not required.	Not Rated	NR	NR	NR
	6. Assay procedures	Assay procedures were described.	High	1	1	1
	7. Standards for tests	No standards required.	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Limited preparation details were reported, but not information about stability and storage were reported.	Medium	2	1	2
	9. Consistency of Exposure Administration	Exposures were administered consistently.	Medium	2	1	2
	10. Reporting of Doses/Concentrations	Concentrations were reported.	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Duration of exposure was reported.	High	1	2	2
	12. Exposure Route and Method	The number of exposure groups and concentration spacing were reported, and the rationale for selected was reported.	High	1	1	1
	13. Metabolic Activation	Metabolic activation not required.	Not Rated	NR	NR	NR
Test Model	14. Test Model	Test model and source information were reported.	High	1	2	2
	15. Number per Group	The number of cells was reported.	High	1	1	1

Study reference:	Anisuzzaman, S.,Whalen, M. M. (2016). Tetrabromobisphenol A and hexabromocyclododecane alter secretion of IL-1 from human immune cells Journal of Immunotoxicology, 13(3), 403-416 **HERO ID: 3350463**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment was reported.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate for the outcomes of interest.	High	1	2	2
	19. Blinding of Assessors	No outcomes required blinding.	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	There were no reported differences among study group parameters.		NR	2	NR
	21. Confounding Variables in Outcomes Unrelated to Exposure	No reported outcome differences among study groups unrelated to exposure were reported.	High	1	1	1
Data Presentation and Analysis	22. Data Analysis	Statistical analysis was reported and appropriate.	High	1	1	1
	23. Data Interpretation	Scoring and evaluation criteria not required.	Not Rated	NR	NR	NR
	24. Cytotoxicity Data	Cell viability was defined and methods were described.	High	1	1	1
	25. Reporting of Data	All data were reported.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**27**	**34**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.2593**	**Overall Score: Nearest *:**	**1.3**
		Overall Quality Level:		**High**		

4.5 In vitro evaluation results of Canbaz et al 2016

Study reference:	**Canbaz, D.,Lebre, M. C.,Logiantara, A.,van Ree, R.,van Rijt, L. S. (2016). Indoor pollutant hexabromocyclododecane enhances house dust mite-induced activation of human monocyte-derived dendritic cells Journal of Immunotoxicology, 13(6), 1-7** **HERO ID: 3355511**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by name.	Medium	2	2	4
	2. Test Substance Source	Source identified.	Medium	2	1	2
	3. Test Substance Purity	Test substance described as technical mixture, but purity/grade and/or composition were not reported.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Concurrent negative controls were used.	High	1	2	2
	5. Positive Controls	Positive controls not required.	Not Rated	NR	NR	NR
	6. Assay procedures	Assay procedures were reported.	High	1	1	1
	7. Standards for tests	Standards not required for assays.	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Limited preparation details were reported, but stability and storage were not.	Medium	2	1	2
	9. Consistency of Exposure Administration	Exposures were administered consistently.	High	1	1	1
	10. Reporting of Doses/Concentrations	Concentrations were administered consistently.	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Durations were reported.	High	1	2	2
	12. Exposure Route and Method	The number of groups and spacing were reported nut not justified.	Medium	2	1	2
	13. Metabolic Activation	Activation not required.	Not Rated	NR	NR	NR
Test Model	14. Test Model	Test model and donor information were provided.	High	1	2	2

Study reference:	**Canbaz, D.,Lebre, M. C.,Logiantara, A.,van Ree, R.,van Rijt, L. S. (2016). Indoor pollutant hexabromocyclododecane enhances house dust mite-induced activation of human monocyte-derived dendritic cells Journal of Immunotoxicology, 13(6), 1-7** **HERO ID: 3355511**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	The number of cells per group in the initial exposure assay was not reported, but was reported for the cytokine assay.	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methodology was described	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate.	High	1	2	2
	19. Blinding of Assessors	Blinding was not required.	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	No confounding variables in test design were observed.	High	1	2	2
	21. Confounding Variables in Outcomes Unrelated to Exposure	Two donors did not yield sufficient cells to perform all experiments.	Medium	2	1	2
Data Presentation and Analysis	22. Data Analysis	Statistical methods were reported and appropriate.	High	1	1	1
	23. Data Interpretation	Data interpretation criteria not required.	Not Rated	NR	NR	NR
	24. Cytotoxicity Data	Methods were not reported but the data were provided.	Low	3	1	3
	25. Reporting of Data	Data were reported.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**40**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.3793**	**Overall Score: Nearest *:**	**1.4**
		Overall Quality Level:		**High**		

4.6 In vitro evaluation results of Ethyl Corporation 1990

Study reference:	**Ethyl Corporation (1990). Genetic toxicology salmonella/microsomal assay on hexabromocyclododecane with cover letter dated 030890** **HERO ID: 787661**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified as HBCD Bottoms, a brittle black solid.	Low	3	2	6
	2. Test Substance Source	Source identified as a person but may have worked for the Sponsor of the study.	Low	3	1	3
	3. Test Substance Purity	Purity was not reported.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Negative and solvent controls were included.	High	1	2	2
	5. Positive Controls	Positive controls were included but were not identified.	Medium	2	2	4
	6. Assay procedures	The methods and procedures were not well described and most details were not reported. Company SOP numbers were reported.	Low	3	1	3
	7. Standards for tests	Criteria were not required.	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Limited details on preparation were reported and no storage details were reported.	Low	3	1	3
	9. Consistency of Exposure Administration		Low	3	1	3
	10. Reporting of Doses/Concentrations	Concentrations were reported.	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Duration was not reported but may be referenced in the SOP documents.	Low	3	2	6
	12. Exposure Route and Method	The number of groups and spacing were reported, but not justified.	Medium	2	1	2
	13. Metabolic Activation	Metabolic activation was reported, but details were not provided.	Medium	2	1	2

Study reference:	Ethyl Corporation (1990). Genetic toxicology salmonella/microsomal assay on hexabromocyclododecane with cover letter dated 030890 **HERO ID: 787661**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Model	14. Test Model	The strains and source were reported, but additional details were not provided	Medium	2	2	4
	15. Number per Group	The number of exposed cells per group was not reported but may be found in the SOP documents.	Low	3	1	3
Outcome Assessment	16. Outcome Assessment Methodology	The outcome assessment methodology was not reported but may be found in the SOP documents.	Low	3	2	6
	17. Consistency of Outcome Assessment	Details were not reported.	Low	3	1	3
	18. Sampling Adequacy	Details were not reported.	Low	3	2	6
	19. Blinding of Assessors	Blinding was not required.	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	Insufficient details were reported to determine.	Low	3	2	6
	21. Confounding Variables in Outcomes Unrelated to Exposure	Insufficient details were reported to determine.	Low	3	1	3
Data Presentation and Analysis	22. Data Analysis	Calculations were not described clearly but inferences could be made.	Low	3	1	3
	23. Data Interpretation	Evaluation criteria were reported.	Medium	2	2	4
	24. Cytotoxicity Data	Cytotoxicity was not reported to have been evaluated.	Not Rated	NR	NR	NR
	25. Reporting of Data	Data were reported.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**33**	**79**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**2.3939**	**Overall Score: Nearest *:**	**2.4**

Study reference:	Ethyl Corporation (1990). Genetic toxicology salmonella/microsomal assay on hexabromocyclododecane with cover letter dated 030890 **HERO ID: 787661**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
		Overall Quality Level:		**Low**		

4.7 In vitro evaluation results of Ethyl Corporation 1990

Study reference:	**Ethyl Corporation (1990). Genetic toxicology rat hepatocyte primary culture/DNR repair test on hexabromocyclododecane with cover letter dated 030890** **HERO ID: 1928253**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified as NBCD Bottoms, brittle black solid.	Medium	2	2	4
	2. Test Substance Source	Source not identified.	Low	3	1	3
	3. Test Substance Purity	Purity not reported.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Solvent control included.	High	1	2	2
	5. Positive Controls	Positive control included.	High	1	2	2
	6. Assay procedures	Assay methods were reported for harvesting cells and data quantifications, but limited details regarding treatment were reported.	Low	3	1	3
	7. Standards for tests	Criteria not required.	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Limited preparation details and no storage details were reported.	Low	3	1	3
	9. Consistency of Exposure Administration	Insufficient data were reported.	Low	3	1	3
	10. Reporting of Doses/Concentrations	Concentrations were reported.	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Durations not reported but may be found in the reported SOP.	Low	3	2	6
	12. Exposure Route and Method	The number of groups and spacing were reported but not justified.	Medium	2	1	2
	13. Metabolic Activation	Metabolic activation was not required.	Not Rated	NR	NR	NR
Test Model	14. Test Model	The test model was described.	High	1	2	2
	15. Number per Group	The number of cells was not reported but may be found in the reported SOP.	Low	3	1	3

Study reference:	Ethyl Corporation (1990). Genetic toxicology rat hepatocyte primary culture/DNR repair test on hexabromocyclododecane with cover letter dated 030890 **HERO ID: 1928253**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	16. Outcome Assessment Methodology	Outcome methodology assessment was reported.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate.	High	1	2	2
	19. Blinding of Assessors	Blinding not required.	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	No confounding variables were reported.	High	1	2	2
	21. Confounding Variables in Outcomes Unrelated to Exposure	No outcomes unrelated to exposure were reported.	High	1	1	1
Data Presentation and Analysis	22. Data Analysis	Statistical methods were reported.	High	1	1	1
	23. Data Interpretation	Criteria for positive or equivocal findings were reported.	High	1	2	2
	24. Cytotoxicity Data	Cytotoxicity not required.	Not Rated	NR	NR	NR
	25. Reporting of Data	Data were reported.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**32**	**51**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.5938**	**Overall Score: Nearest *:**	**1.6**
		Overall Quality Level:		**High**		

4.8 In vitro evaluation results of Huang et al 2016

Study reference:	**Huang, X.,Chen, C.,Shang, Y.,Zhong, Y.,Ren, G.,Yu, Z.,An, J. (2016). In vitro study on the biotransformation and cytotoxicity of three hexabromocyclododecane diastereoisomers in liver cells Chemosphere, 161, 251-258** **HERO ID: 3545979**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Native-HBCDs and isomers were named	Medium	2	2	4
	2. Test Substance Source	Source provided but no other details were reported	Medium	2	1	2
	3. Test Substance Purity	Uncertainty of purity was present, however, given that unlabeled and labeled products were used in the studies, any effects observed are more likely than not to be due to the test substance	Medium	2	1	2
Test Design	4. Negative and Vehicle Controls	Concurrent negative controls were used	High	1	2	2
	5. Positive Controls	Positive controls not required for these assays	Not Rated	NR	NR	NR
	6. Assay procedures	Assays procedures were described in detail	High	1	1	1
	7. Standards for tests	Not applicable for these assays	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Limited details were reported regarding stock solution preparation and no details were reported regarding storage.	Medium	2	1	2
	9. Consistency of Exposure Administration	Consistency of administration was reported	High	1	1	1
	10. Reporting of Doses/Concentrations	Concentrations were reported	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Durations were reported for each assay	High	1	2	2
	12. Exposure Route and Method	Number of groups and concentration spacing were appropriate	High	1	1	1
	13. Metabolic Activation	Metabolic activation not required for these assays	Not Rated	NR	NR	NR

Study reference:	Huang, X.,Chen, C.,Shang, Y.,Zhong, Y.,Ren, G.,Yu, Z.,An, J. (2016). In vitro study on the biotransformation and cytotoxicity of three hexabromocyclododecane diastereoisomers in liver cells Chemosphere, 161, 251-258 **HERO ID: 3545979**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Model	14. Test Model	The cell types used were appropriate for the intended outcomes	High	1	2	2
	15. Number per Group	Limited information on the number of cells were reported, number of replicates were reported for each assay,	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methodologies were described in detail	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes assessments were conducted consistently	High	1	1	1
	18. Sampling Adequacy	Details regarding sampling outcomes were not fully reported.	Medium	2	2	4
	19. Blinding of Assessors	Blinding was not applicable for this study	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	No confounding variables were reported	High	1	2	2
	21. Confounding Variables in Outcomes Unrelated to Exposure	No confounding variables were reported	High	1	1	1
Data Presentation and Analysis	22. Data Analysis	Statistical analysis was appropriate	High	1	1	1
	23. Data Interpretation	Not applicable for the assays	Not Rated	NR	NR	NR
	24. Cytotoxicity Data	Cytotoxicity was defined and methods were described sufficiently	High	1	1	1
	25. Reporting of Data	Outcome data were reported	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**37**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.2759**	**Overall Score: Nearest *:**	**1.3**
		Overall Quality Level:		**High**		

4.9 In vitro evaluation results of Kim et al 2016

Study reference:	**Kim, S. H.,Nam, K. H.,Hwang, K. A.,Choi, K. C. (2016). Influence of hexabromocyclododecane and 4-nonylphenol on the regulation of cell growth, apoptosis and migration in prostatic cancer cells Toxicology In Vitro, 32, 240-247** **HERO ID: 3350494**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Established nomenclature used	High	1	2	2
	2. Test Substance Source	Source identified, but no additional information provided	Medium	2	1	2
	3. Test Substance Purity	Purity such that effects likely due to test substance	High	1	1	1
Test Design	4. Negative and Vehicle Controls	A concurrent vehicle control was used	High	1	2	2
	5. Positive Controls	A concurrent positive control was used	High	1	2	2
	6. Assay procedures	Experimental procedures were described	High	1	1	1
	7. Standards for tests	Not applicable for this study	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Test substance was dissolved in solvent but no other details were provided	Medium	2	1	2
	9. Consistency of Exposure Administration	No deficiencies noted	High	1	1	1
	10. Reporting of Doses/Concentrations	Concentrations were reported	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Exposure durations are listed for all experiments	High	1	2	2
	12. Exposure Route and Method	The number of groups and spacing are appropriate for these study types	High	1	1	1
	13. Metabolic Activation	Metabolic activation not required	Not Rated	NR	NR	NR
Test Model	14. Test Model	Test model was appropriate	High	1	2	2
	15. Number per Group	The number of cells and replicates were appropriate	High	1	1	1

Study reference:	**Kim, S. H.,Nam, K. H.,Hwang, K. A.,Choi, K. C. (2016). Influence of hexabromocyclododecane and 4-nonylphenol on the regulation of cell growth, apoptosis and migration in prostatic cancer cells Toxicology In Vitro, 32, 240-247** **HERO ID: 3350494**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	16. Outcome Assessment Methodology	Methodology for outcome assessment was reported in detail	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently across groups	High	1	1	1
	18. Sampling Adequacy	See footnote at end of page.[4]	High	1	2	2
	19. Blinding of Assessors	No subjective outcomes were assessed	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	No confounding variables were reported.	High	1	2	2
	21. Confounding Variables in Outcomes Unrelated to Exposure	No confounding variables were reported	High	1	1	1
Data Presentation and Analysis	22. Data Analysis	Appropriate statistical tests were used	High	1	1	1
	23. Data Interpretation	This metric is not applicable for these studies	Not Rated	NR	NR	NR
	24. Cytotoxicity Data	Methods to determine cell viability were described	High	1	1	1
	25. Reporting of Data	Data were reported for all outcomes	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**31**	**33**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.0645**	**Overall Score: Nearest *:**	**1.1**
		Overall Quality Level:		**High**		

[4] Metrics that received a "High" rating met the criteria as discussed in the Applications of Systematic Review for TSCA Risk Evaluation.

4.10 In vitro evaluation results of Koike et al 2016

Study reference:	**Koike, E.,Yanagisawa, R.,Takano, H. (2016). Brominated flame retardants, hexabromocyclododecane and tetrabromobisphenol A, affect proinflammatory protein expression in human bronchial epithelial cells via disruption of intracellular signaling Toxicology In Vitro, 32, 212-219** **HERO ID: 3350501**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by name, structure, and molecular weight.	High	1	2	2
	2. Test Substance Source	The source was identified.	Medium	2	1	2
	3. Test Substance Purity	The reported purity was such that observed effects are likely due to the test substance.	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Concurrent negative controls were included.	High	1	2	2
	5. Positive Controls	Positive controls not required.	Not Rated	NR	NR	NR
	6. Assay procedures	Assay procedures were reported.	High	1	1	1
	7. Standards for tests	Metric not required for the test.	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Preparation details were reported, but storage and stability were not reported.	Medium	2	1	2
	9. Consistency of Exposure Administration	Exposures were administered consistently.	High	1	1	1
	10. Reporting of Doses/Concentrations	Concentrations were reported (found in graphs and/or figure legends).	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Durations were reported, but ranges were given for the transcription assay, and no duration was given for the ligand-binding assay.	Medium	2	2	4
	12. Exposure Route and Method	The number of groups was provided for each assay, but spacing was not justified.	Medium	2	1	2
	13. Metabolic Activation	Metabolic activation was not required.	Not Rated	NR	NR	NR
Test Model	14. Test Model	Test model and information were provided.	High	1	2	2

Study reference:	**Koike, E.,Yanagisawa, R.,Takano, H. (2016). Brominated flame retardants, hexabromocyclododecane and tetrabromobisphenol A, affect proinflammatory protein expression in human bronchial epithelial cells via disruption of intracellular signaling Toxicology In Vitro, 32, 212-219** **HERO ID: 3350501**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	The number of cells and replicates used was reported.	High	1	1	1
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methodology was described and appropriate.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate.	High	1	2	2
	19. Blinding of Assessors	Blinding not required.	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	No confounding variable in assay design were reported.	High	1	2	2
	21. Confounding Variables in Outcomes Unrelated to Exposure	No confounding variables in outcomes were reported.	High	1	1	1
Data Presentation and Analysis	22. Data Analysis	Statistical methods were appropriate.	High	1	1	1
	23. Data Interpretation	This metric not applicable.	Not Rated	NR	NR	NR
	24. Cytotoxicity Data	Cell viability endpoints were described and appropriate.	High	1	1	1
	25. Reporting of Data	Data were reported.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**34**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.1724**	**Overall Score: Nearest *:**	**1.2**
		Overall Quality Level:		**High**		

4.11 In vitro evaluation results of Litton et al 1990

Study reference:	**Litton Bionetics (1990). Mutagenicity evaluation of 421-32B (final report) with test data and cover letter HERO ID: 787698**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance was identified by name, and CASE# was hand-written on title page.	Medium	2	2	4
	2. Test Substance Source	Source not identified.	Low	3	1	3
	3. Test Substance Purity	Purity was not reported.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Solvent controls were included.	High	1	2	2
	5. Positive Controls	Positive controls were included and identified.	High	1	2	2
	6. Assay procedures	Assay procedures were reported.	High	1	1	1
	7. Standards for tests	Criteria not applicable	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Preparation was reported with missing details (i.e., solvent used) and storage information was not reported.	Low	3	1	3
	9. Consistency of Exposure Administration	Exposures were administered consistently.	High	1	1	1
	10. Reporting of Doses/Concentrations	Concentrations were reported.	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Exposure durations were reported.	High	1	2	2
	12. Exposure Route and Method	The number of groups and spacing were reported and justified.	High	1	1	1
	13. Metabolic Activation	The system and reaction mixture were reported.	High	1	1	1
Test Model	14. Test Model	The strains were reported, but no additional information was reported.	Low	3	2	6

Study reference:	Litton Bionetics (1990). Mutagenicity evaluation of 421-32B (final report) with test data and cover letter **HERO ID: 787698**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	The number of cells exposed was reported and adequate but the number of replicates was not reported.	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methodology was reported and appropriate.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate.	High	1	2	2
	19. Blinding of Assessors	Blinding was not required.	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	No confounding variables were reported.	High	1	2	2
	21. Confounding Variables in Outcomes Unrelated to Exposure	No outcomes unrelated to exposure were reported.	High	1	1	1
Data Presentation and Analysis	22. Data Analysis	Calculation and/or statistical methods were not reported, but the data was present to conduct analysis.	Low	3	1	3
	23. Data Interpretation	Evaluation criteria were not reported.	Low	3	2	6
	24. Cytotoxicity Data	Description of cytotoxicity wans how it was determined was not reported.	Low	3	1	3
	25. Reporting of Data	Data were reported	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**34**	**55**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.6176**	**Overall Score: Nearest *:**	**1.6**
		Overall Quality Level:		**High**		

4.12 In vitro evaluation results of Pharmakologisches et al 1990

Study reference:	**Pharmakologisches Inst (1990). Ames test with hexabromides with cover letter dated 031290** **HERO ID: 787701**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by chemical name and structure.	High	1	2	2
	2. Test Substance Source	Source not identified.	Low	3	1	3
	3. Test Substance Purity	Reported purity such that effects likely due to test substance.	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Negative controls were included .	High	1	2	2
	5. Positive Controls	Appropriate positive controls were included.	High	1	2	2
	6. Assay procedures	Assay procedures were reported.	High	1	1	1
	7. Standards for tests	Criteria were not required.	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Preparation details were reported, but no additional details were provided such as covering and storage conditions prior to assay.	Medium	2	1	2
	9. Consistency of Exposure Administration	Exposures were administered consistently.	High	1	1	1
	10. Reporting of Doses/Concentrations	Concentrations were reported.	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Exposure duration was reported.	High	1	2	2
	12. Exposure Route and Method	The number of exposure groups and spacing were reported but were not justified.	Medium	2	1	2
	13. Metabolic Activation	The metabolic activation system and mix were reported.	High	1	1	1
Test Model	14. Test Model	Test models were reported without additional details.	Medium	2	2	4

Study reference:	Pharmakologisches Inst (1990). Ames test with hexabromides with cover letter dated 031290 **HERO ID: 787701**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	The number of cells used was reported, but the number of replicates was not.	Medium	2	1	2
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methodology was reported.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate.	High	1	2	2
	19. Blinding of Assessors	Counters were blinded.	High	1	1	1
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	No confounding variables were reported.	High	1	2	2
	21. Confounding Variables in Outcomes Unrelated to Exposure	No outcomes unrelated to exposure were reported.	High	1	1	1
Data Presentation and Analysis	22. Data Analysis	Data were not analyzed but were presented so analysis can be conducted if needed.	Medium	2	1	2
	23. Data Interpretation	Criteria were not reported.	Low	3	2	6
	24. Cytotoxicity Data	Cytotoxicity was not included in the study.	Not Rated	NR	NR	NR
	25. Reporting of Data	Data were reported.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**34**	**46**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.3529**	**Overall Score: Nearest *:**	**1.4**
		Overall Quality Level:		**High**		

4.13 In vitro evaluation results of S.R.I. International 1990

Study reference:	**S. R. I. International (1990). In vitro microbiological mutagenicity studies of four Ciba-Geigy Corporation compounds (final report) with test data and cover letter** **HERO ID: 787716**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by name, CAS# on title page.	Medium	2	2	4
	2. Test Substance Source	Source not identified, but compound was called "Ciby-Geigy Corporation compounds"	Medium	2	1	2
	3. Test Substance Purity	Purity was not reported.	Low	3	1	3
Test Design	4. Negative and Vehicle Controls	Negative controls were included.	High	1	2	2
	5. Positive Controls	Positive controls were included.	High	1	2	2
	6. Assay procedures	Assay procedures were partially described.	Medium	2	1	2
	7. Standards for tests	Criteria were not required.	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Preparation was reported; substances used immediately after preparation.	High	1	1	1
	9. Consistency of Exposure Administration	Exposures were administered consistently.	High	1	1	1
	10. Reporting of Doses/Concentrations	Concentrations were reported.	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Durations were reported.	High	1	2	2
	12. Exposure Route and Method	The number of groups and spacing were reported but not justified.	Medium	2	1	2
	13. Metabolic Activation	The metabolic system and mix were described.	High	1	1	1
Test Model	14. Test Model	Test model, source, and descriptive information was reported.	High	1	2	2

Study reference:	S. R. I. International (1990). In vitro microbiological mutagenicity studies of four Ciba-Geigy Corporation compounds (final report) with test data and cover letter **HERO ID: 787716**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	The number of exposed cells was not reported, but cells were shaken for 3-4 hours to ensure optimal growth.	Low	3	1	3
Outcome Assessment	16. Outcome Assessment Methodology	Outcome methodology assessment was reported.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate.	High	1	2	2
	19. Blinding of Assessors	Blinding was not required.	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	No confounding variables were reported.	High	1	2	2
	21. Confounding Variables in Outcomes Unrelated to Exposure	No outcomes unrelated to exposure were reported.	High	1	1	1
Data Presentation and Analysis	22. Data Analysis	Data were provided to conduct analysis.	Medium	2	1	2
	23. Data Interpretation	Evaluation criteria were not reported.	Low	3	2	6
	24. Cytotoxicity Data	Cytotoxicity not included in test.	Not Rated	NR	NR	NR
	25. Reporting of Data	Data were reported.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**33**	**47**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.4242**	**Overall Score: Nearest *:**	**1.4**
		Overall Quality Level:		**High**		

4.14 In vitro evaluation results of Wang et al 2016

Study reference:	**Wang, F.,Zhang, H.,Geng, N.,Zhang, B.,Ren, X.,Chen, J. (2016). New Insights into the Cytotoxic Mechanism of Hexabromocyclododecane from a Metabolomic Approach Environmental Science and Technology, 50(6), 3145-3153** **HERO ID: 3350479**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by name.	Medium	2	2	4
	2. Test Substance Source	Source identified.	Medium	2	1	2
	3. Test Substance Purity	Reported purity and grade such that effects likely due to test substance.	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Negative control groups were included.	High	1	2	2
	5. Positive Controls	Positive control groups were not required.	Not Rated	NR	NR	NR
	6. Assay procedures	Assay procedures were described.	High	1	1	1
	7. Standards for tests	Standards not applicable for the assay.	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Limited preparation details were provided, but no storage and stability information were provided.	Medium	2	1	2
	9. Consistency of Exposure Administration	Exposures were administered consistently.	High	1	1	1
	10. Reporting of Doses/Concentrations	Concentrations were reported.	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Durations were reported.	High	1	2	2
	12. Exposure Route and Method	The number of groups and spacing were reported and based on cell viability testing.	High	1	1	1
	13. Metabolic Activation	Metabolic activation was not required.	Not Rated	NR	NR	NR
Test Model	14. Test Model	The information was reported.	High	1	2	2
	15. Number per Group	The number of cells exposed was reported.	High	1	1	1

Study reference:	**Wang, F.,Zhang, H.,Geng, N.,Zhang, B.,Ren, X.,Chen, J. (2016). New Insights into the Cytotoxic Mechanism of Hexabromocyclododecane from a Metabolomic Approach Environmental Science and Technology, 50(6), 3145-3153** **HERO ID: 3350479**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment was described.	High	1	2	2
	17. Consistency of Outcome Assessment	Exposures were administered consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate.	High	1	2	2
	19. Blinding of Assessors	Blinding not required.	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	No confounding variables in test design and procedures were reported.	High	1	2	2
	21. Confounding Variables in Outcomes Unrelated to Exposure	No confounding variables in outcomes unrelated to exposure were reported.	High	1	1	1
Data Presentation and Analysis	22. Data Analysis	Statistical methods and data manipulation were reported and appropriate.	High	1	1	1
	23. Data Interpretation	Data evaluation not required.	Not Rated	NR	NR	NR
	24. Cytotoxicity Data	Cytotoxicity studies were described.	High	1	1	1
	25. Reporting of Data	Data were reported for outcomes.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**29**	**33**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.1379**	**Overall Score: Nearest *:**	**1.1**
		Overall Quality Level:		**High**		

4.15 In vitro evaluation results of Wu et al 2016

Study reference:	**Wu, M.,Wu, D.,Wang, C.,Guo, Z.,Li, B.,Zuo, Z. (2016). Hexabromocyclododecane exposure induces cardiac hypertrophy and arrhythmia by inhibiting miR-1 expression via up-regulation of the homeobox gene Nkx2.5 Journal of Hazardous Materials, 302, 304-313** **HERO ID: 3350515**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Test Substance	1. Test Substance Identity	Test substance identified by structure and name.	High	1	2	2
	2. Test Substance Source	The source was identified.	Medium	2	1	2
	3. Test Substance Purity	The reported purity was such that effects likely due to the test substance.	High	1	1	1
Test Design	4. Negative and Vehicle Controls	Concurrent controls were included.	High	1	2	2
	5. Positive Controls	Positive controls not required.	Not Rated	NR	NR	NR
	6. Assay procedures	Assay procedures were described and appropriate.	High	1	1	1
	7. Standards for tests	Metric not applicable to study type.	Not Rated	NR	NR	NR
Exposure Characterization	8. Preparation and Storage of Test Substance	Limited preparation details were reported and no details on storage and stability were reported.	Medium	2	1	2
	9. Consistency of Exposure Administration	Exposures were administered consistently.	High	1	1	1
	10. Reporting of Doses/Concentrations	Concentrations were reported.	High	1	2	2
	11. Number of Exposure Groups and Concentration Spacing	Duration was reported.	High	1	2	2
	12. Exposure Route and Method	The number of groups and spacing were reported, but justification was not provided.	Medium	2	1	2
	13. Metabolic Activation	Metabolic activation was not required.	Not Rated	NR	NR	NR
Test Model	14. Test Model	The test model and source were reported and were appropriate.	High	1	2	2

Study reference:	**Wu, M.,Wu, D.,Wang, C.,Guo, Z.,Li, B.,Zuo, Z. (2016). Hexabromocyclododecane exposure induces cardiac hypertrophy and arrhythmia by inhibiting miR-1 expression via up-regulation of the homeobox gene Nkx2.5 Journal of Hazardous Materials, 302, 304-313** **HERO ID: 3350515**					
Domain	**Metric**	**Evaluator's Comment**	**Qualitative Determination [i.e.,High,Medium, Low,Unacceptable, or Not rated]**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	15. Number per Group	n-6 was reported in the figures, but it is not clear if that is the number of replicates. The number of cells used was not reported.	Low	3	1	3
Outcome Assessment	16. Outcome Assessment Methodology	Outcome assessment methodology was reported and appropriate.	High	1	2	2
	17. Consistency of Outcome Assessment	Outcomes were assessed consistently.	High	1	1	1
	18. Sampling Adequacy	Sampling was adequate.	High	1	2	2
	19. Blinding of Assessors	Blinding was not required.	Not Rated	NR	NR	NR
Confounding / Variable Control	20. Confounding Variables in Test Design and Procedures	No confounding variables in test design were reported.	High	1	2	2
	21. Confounding Variables in Outcomes Unrelated to Exposure	No confounding variables in outcomes unrelated to exposure were reported.	High	1	1	1
Data Presentation and Analysis	22. Data Analysis	Statistical methods were appropriate.	High	1	1	1
	23. Data Interpretation	This metric not applicable.	Not Rated	NR	NR	NR
	24. Cytotoxicity Data	This metric not applicable.	Not Rated	NR	NR	NR
	25. Reporting of Data	Data were reported.	High	1	2	2
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**28**	**33**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.1786**	**Overall Score: Nearest *:**	**1.2**
		Overall Quality Level:		**High**		

5 Epidemiological Studies

5.1 Epidemiological evaluation results of the Eggesbø et al 2011 study for thyroid outcomes for exposure groups in general

Study reference:	**Eggesbø, M.,Thomsen, C.,Jørgensen, J. V.,Becher, G.,Odland, J. Ø,Longnecker, M. P. (2011). Associations between brominated flame retardants in human milk and thyroid-stimulating hormone (TSH) in neonates Environmental Research, 111(6), 737-743** **HERO ID: 787656**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Study Participation	1. Participant selection	High rating: key elements of study design were reported (such as setting, participation rate described at all steps of the study, inclusion and exclusion criteria, and methods of participant selection), and the reported information indicates selection in or out of the study and participation is not likely to be biased.	High	1	0.400	0.400
	2. Attrition	Medium rating: 31% of women that agreed to participate in the study did not provide milk samples (authors explained this was partly due to lack of milk); 40% of the 396 babies selected for the study were excluded from analysis due to inaccessible TSH values. Attrition was acceptably handled. Supplemental Fig A1 provides a description of characteristics between participants and non-participants. No significant differences were reported between these 2 groups. Missing values for ‘‘age at which TSH was measured’’ were replaced by mean values for 80 (33%) participants.	Medium	2	0.400	0.800
	3. Comparison Group	High rating: differences in baseline characteristics of groups were considered as potential confounding or stratification variables and were thereby controlled by statistical analysis. Covariates included age at which TSH was measured(continuously in hours), county of residence and pre-pregnancy maternal body mass index. The following potential confounders: maternal education as a socioeconomic index (<12, 12, 13–16 and >16 years of education), Norwegian nationality, season, parity, smoking, maternal age at delivery, sex, pregnancy hypertension and/or preeclampsia based on maternal reports (yes/no) and type of delivery (spontaneous, induced, assisted or cesarean); and continuous variables: gestational age, HCB, b-HCH,p,p0-DDE,oxychlordane and the sum of all PCB congeners.	High	1	0.200	0.200
Exposure Characterization	4. Measurement of Exposure	High rating: exposure was assessed using the same well-established methods that directly measure HBCD in breast milk, a frequently used biomarker of exposure.	High	1	0.400	0.400

Study reference:	**Eggesbø, M.,Thomsen, C.,Jørgensen, J. V.,Becher, G.,Odland, J. Ø,Longnecker, M. P. (2011). Associations between brominated flame retardants in human milk and thyroid-stimulating hormone (TSH) in neonates Environmental Research, 111(6), 737-743** **HERO ID: 787656**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	5. Exposure levels	Medium rating: range and distribution of exposure was sufficient to develop an exposure-response estimate; 3 or more levels of exposure were reported.	Medium	2	0.200	0.400
	6. Temporality	High rating: temporality is established and the interval between the exposure and the outcome has an appropriate consideration of relevant exposure windows.	High	1	0.400	0.400
Outcome Assessment	7. Outcome measurement or characterization	High rating: TSH levels were measured using well-established methods (i.e., on dried filter paper bloodspots by an immunoassay) (Auto Delfias neonatal TSH kits; Perkin Elmer).	High	1	0.670	0.667
	8. Reporting Bias	High rating: all of the study's measured outcomes are reported, effect estimates reported with confidence interval; number of exposed reported for each analysis.	High	1	0.330	0.333
Potential Confounding/Variable Control	9. Covariate Adjustment	High rating: appropriate adjustments or explicit considerations were made for potential confounders in the final analyses through the use of statistical models for covariate adjustment. See discussion in metric 3.	High	1	0.500	0.500
	10. Covariate Characterization	Medium rating: Primary confounders (excluding co-exposures) were assessed. The paper did not describe if the survey to gather demographic characteristics, the amount of breastfeeding/month, etc. was validated.	Medium	2	0.250	0.500
	11. Co-exposure Confounding	Medium rating: HBCD models were adjusted for some co-pollutants (PCBs, HCB, DDE, etc); however, separate models were run for PBDEs and HBCD, and it difficult to distinguish which contaminant might have caused an association with a disease. However, there does not appear to be direct evidence of an unbalanced provision of additional co-exposures across the primary study groups,	Medium	2	0.250	0.500
Analysis	12. Study Design and Methods	Medium rating: appropriate design (i.e., prospective cohort for assessment of TSH levels in relation to HBCD exposure), and appropriate statistical methods (i.e., linear and logistic regression analyses) were employed to analyze data.	Medium	2	0.400	0.800

Study reference:	**Eggesbø, M.,Thomsen, C.,Jørgensen, J. V.,Becher, G.,Odland, J. Ø,Longnecker, M. P. (2011). Associations between brominated flame retardants in human milk and thyroid-stimulating hormone (TSH) in neonates Environmental Research, 111(6), 737-743** **HERO ID: 787656**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	13. Statistical power	Medium rating: the number of participants were adequate to detect an effect in the exposed population for HBCD and for most BFRs except BDE- 209.	Medium	2	0.200	0.400
	14. Reproducibility of analyses	Medium rating: description of the analyses is sufficient to understand what has been done and to be reproducible with access to the data.	Medium	2	0.200	0.400
	15. Statistical models	Medium rating: linear regression models were used to generate beta coefficients and logistic regression models were used to generate Odds Ratios. Rationale for variable selection is stated. Model assumptions are met.	Medium	2	0.200	0.400
Other	16. Use of Biomarker of Exposure	High rating: Evidence exists for a relationship between HBCD in breast milk and external exposure.	High	1	0.140	0.143
	17. Effect biomarker	High rating: Effect biomarker measured is an indicator of a key event in an AOP.	High	1	0.140	0.143
	18. Method Sensitivity	Medium rating: LOD is low enough to detect HBCD in a sufficient percentage of the samples to address the research question. Analytical methods measuring biomarker are adequately reported. LOD/LOQ (value or %) are reported.	Medium	2	0.140	0.286
Other	19. Biomarker stability	High rating: samples with a known storage history (Supplement-03 document)	High	1	0.140	0.140
	20. Sample contamination	Low rating: No known sampling contamination issues are discussed in the paper, but there is no documentation of the steps taken to provide the necessary assurance that the study data are reliable.	Low	3	0.140	0.429

Study reference:	**Eggesbø, M.,Thomsen, C.,Jørgensen, J. V.,Becher, G.,Odland, J. Ø,Longnecker, M. P. (2011). Associations between brominated flame retardants in human milk and thyroid-stimulating hormone (TSH) in neonates Environmental Research, 111(6), 737-743** **HERO ID: 787656**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Other	21. Method requirements	High rating: instrumentation that provides unambiguous identification and quantitation of the biomarker at the required sensitivity were used. Specifically, the extracts were analyzed by gas chromatography coupled to a mass spectrometer using electron capture negative ionization (GC- EC/MS) and an internal standard calibration as described by Thomsen et al., 2007.	High	1	0.140	0.143
	22. Matrix adjustment	Medium rating: study only provides results using one method (lipid-adjusted).	Medium	2	0.140	0.286
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**6**	**8.53**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.4217**	**Overall Score: Nearest *:**	**1.4**
		Overall Quality Level:		**High**		

5.2 Epidemiological evaluation results of the Johnson et al 2013 study for reproductive outcomes in general

Study reference:	**Johnson, P. I.,Stapleton, H. M.,Mukherjee, B.,Hauser, R.,Meeker, J. D. (2013). Associations between brominated flame retardants in house dust and hormone levels in men Science of the Total Environment, 445-446(Supplement C), 177-184** **HERO ID: 1676758**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Study Participation	1. Participant selection	No explanation for participation rate of 65% provided; only male subjects. Information on participation selection, inclusion and exclusion criteria are provided in cited publications.	High	1	0.250	0.250
	2. Attrition	Attrition is not reported, and n values do not equal 62 in all results presented. (e.g. T3 has n=38 which is ~40% missing samples). No information on how missing data is handled.	Low	3	0.250	0.750
	3. Comparison Group	There is no information on a comparison group, however correlation analysis was performed looking for trends on a continuum of exposure.	Unacceptable	NR	NR	NR
Exposure Characterization	4. Measurement of Exposure	Dust samples were collected from used vacuum badge from home. It is unclear if this is an established method to determine levels of exposure. HBCD detected in 97% of samples.	Medium	2	0.400	0.800
	5. Exposure levels	The range of exposure is limited but based on the analysis it does allow limited exploration in the exposure-response relationship.	Low	3	0.200	0.600
	6. Temporality	Dust samples and serum hormone levels are sampled in the same year for participants. The temporality of exposure and outcome is uncertain.	Medium	2	0.400	0.800
Outcome Assessment	7. Outcome measurement or characterization	QA/QC methods described in another paper. The outcome was assessed using established methods.	High	1	0.670	0.667
	8. Reporting Bias	Author's discuss results in text for significant results only	Medium	2	0.330	0.667
Potential Confounding/ Variable Control	9. Covariate Adjustment	Although models were adjusted for age and BMI for some flame retardants, there is no mention of covariate consideration for HBCD.	High	1	0.500	0.500

Study reference:	Johnson, P. I.,Stapleton, H. M.,Mukherjee, B.,Hauser, R.,Meeker, J. D. (2013). Associations between brominated flame retardants in house dust and hormone levels in men Science of the Total Environment, 445-446(Supplement C), 177-184 HERO ID: 1676758					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	10. Covariate Characterization	There is no information to suggest that the questionnaire used was validated, however there is no evidence that the method had poor validity.	High	1	0.250	0.250
	11. Co-exposure Confounding	Cannot rule out possibility of that findings are due to unmeasured co-exposures (e.g. other chemicals in household dust).	Medium	2	0.250	0.500
Analysis	12. Study Design and Methods	The study was exploratory to assess the association between exposure levels and hormone levels. However only a correlation analysis between HBCD and free androgen index was reported.	Unacceptable	NR	NR	NR
	13. Statistical power	The sample size is relatively small and the authors indicate that the study is exploratory in nature.	Unacceptable	NR	NR	NR
	14. Reproducibility of analyses	The analysis is sufficiently described.	Medium	2	0.070	0.143
	15. Statistical models	The authors provide an explanation for when data is combined with previous study data and limitations of the analysis in detail.	Medium	2	0.070	0.143
Other	16. Use of Biomarker of Exposure	No biomarker of exposure measured.	Not Rated	NR	NR	NR
	17. Effect biomarker	Biomarker not specific to a health outcome.	Unacceptable	NR	NR	NR

Study reference:	Johnson, P. I.,Stapleton, H. M.,Mukherjee, B.,Hauser, R.,Meeker, J. D. (2013). Associations between brominated flame retardants in house dust and hormone levels in men Science of the Total Environment, 445-446(Supplement C), 177-184 HERO ID: 1676758					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	18. Method Sensitivity	Limit of detection not discussed in study, but no evidence of insufficient data.	Not Rated	NR	NR	NR
Other	19. Biomarker stability	samples with known storage history and documented stability data	High	NR	NR	NR
	20. Sample contamination	No information to indicate sample contamination.	Medium	2	0.140	0.286
Other	21. Method requirements	Method provides the identification and quantification of the biomarker.	High	1	0.140	0.143
	22. Matrix adjustment	No matrix adjustment.	Not Rated	NR	NR	NR
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**4.07**	**6.5**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**2.5273**	**Overall Score: Nearest *:**	**2.5[1]**
		Overall Quality Level:		**Unacceptable[1]**		
Comment:	**Footnote:** **[1] Consistent with our *Application of Systematic Review in TSCA Risk Evaluations document*, if a metric for a data source receives a score of Unacceptable (score = 4), EPA will determine the study to be unacceptable. In this case, four of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.**					

5.3 Epidemiological evaluation results of the Kicinski et al 2012 study for neurological/behavior outcomes in general

Study reference:	**Kicinski, M.,Viaene, M. K.,Den Hond, E.,Schoeters, G.,Covaci, A.,Dirtu, A. C.,Nelen, V.,Bruckers, L.,Croes, K.,Sioen, I.,Baeyens, W.,Van Larebeke, N.,Nawrot, T. S. (2012). Neurobehavioral function and low-level exposure to brominated flame retardants in adolescents: A cross-sectional study Environmental Health: A Global Access Science Source, 11(#issue#), 86** **HERO ID: 1927571**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Study Participation	1. Participant selection	Participants were recruited during the same time frame (2008-2011) from the same two industrial areas and from the general population of Flemish adolescents using the same criteria. All adolescents from Genk and Menen were eligible. Random sampling of the general population was attained through a multistage sampling design (which is described). Details were provided for all aspects of the selection. The response rates were slightly higher in Genk, but non-responders were noted to not be different from the responders except that there was a higher proportion of girls responding.	High	1	0.400	0.400
	2. Attrition	107 of the 606 subjects included were excluded because of missing covariates (n=84), missing blood measurements (n=3), or did not complete neuro-behavioral tests (n=4). However, results have much fewer numbers for some results without full explanation.	Medium	2	0.400	0.800
	3. Comparison Group	Although a table of characteristics was provided, it was not broken down by area or general population. Differences that were expected to potentially bias the results were included in the analysis. However, there is not enough information provided about the two study areas to determine if there may have been other differences that varied by exposure.	Medium	2	0.200	0.400
Exposure Characterization	4. Measurement of Exposure	HBCD was measured in the serum according to methods by Covaci and Voorspoels (HERO ID 3113586). However, the method they cite does not indicate that this is a method for measuring HBCD nor do they provide recovery rates. Despite that there is no evidence that there would be poor validity or misclassification, it may just be more likely that samples would fall below the LOQ.	Low	3	0.400	1.200
	5. Exposure levels	For HBCD the effects of the concentrations above the LOQ compared to the concentrations below the LOQ were estimated (binary exposure).	Low	3	0.200	0.600
	6. Temporality	The temporality of exposure and outcome is uncertain . The cross-sectional nature of the study design makes it difficult to determine if exposure occurred prior to the outcome.	Low	3	0.400	1.200

Study reference:	Kicinski, M.,Viaene, M. K.,Den Hond, E.,Schoeters, G.,Covaci, A.,Dirtu, A. C.,Nelen, V.,Bruckers, L.,Croes, K.,Sioen, I.,Baeyens, W.,Van Larebeke, N.,Nawrot, T. S. (2012). Neurobehavioral function and low-level exposure to brominated flame retardants in adolescents: A cross-sectional study Environmental Health: A Global Access Science Source, 11(#issue#), 86 HERO ID: 1927571					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	7. Outcome measurement or characterization	Neurobehavioral Evaluation System is a computerized battery of tests developed to study the neurological effects of an exposure to environmental agents. This study used four tests from the NES-3 version of the test battery. Study authors note these tests are reliable.	High	1	0.670	0.667
	8. Reporting Bias	Sufficient information is provided. All outcomes were reported with effect, 95% confidence intervals, and sample size.	High	1	0.330	0.333
Potential Confounding/Variable Control	9. Covariate Adjustment	Gender, age, type of education, parental education, owning the house, smoking , passive smoking, and blood lipids were included in the assessment. BMI, physical activity, computer use, alcohol use, fish consumption, blood lead, serum PCBs were also included in a stepwise regression procedure with p=0.15 for entering and p=0.10 for remaining in the model.	High	1	0.500	0.500
	10. Covariate Characterization	Information was obtained via questionnaires some information to be filled out by the adolescent and some for the parents.	Medium	2	0.250	0.500
	11. Co-exposure Confounding	Two of the groups were selected because they lived near industrial areas. No information was provided on these industrial areas and what else might be there. However, they did account for lead and PCBs (and possibly mercury via fish consumption) because these may impact the results. Although it is unclear if there might be other potential co-exposures, there is no indication that there would be anything additional that would greatly impact the results that was not considered.	Medium	2	0.250	0.500
Analysis	12. Study Design and Methods	The cross-sectional study design is appropriate for evaluating HBCD concentrations in adolescents with neurobehavioral effects. The study was part of a biomonitoring program for environmental health surveillance in Flanders, Belgium.	Medium	2	0.400	0.800
	13. Statistical power	Sufficient statistical power with 515 included subjects and outcome results available for 340 to 511 for any specific outcome.	Medium	2	0.200	0.400

Study reference:	**Kicinski, M.,Viaene, M. K.,Den Hond, E.,Schoeters, G.,Covaci, A.,Dirtu, A. C.,Nelen, V.,Bruckers, L.,Croes, K.,Sioen, I.,Baeyens, W.,Van Larebeke, N.,Nawrot, T. S. (2012). Neurobehavioral function and low-level exposure to brominated flame retardants in adolescents: A cross-sectional study Environmental Health: A Global Access Science Source, 11(#issue#), 86** **HERO ID: 1927571**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	14. Reproducibility of analyses	Description is not 100% clear on methods to be reproducible.	Low	3	0.200	0.600
	15. Statistical models	The use of a linear regression or a negative binomial model were acceptable for the data with assumptions met or data transformed.	Medium	2	0.200	0.400
Other	16. Use of Biomarker of Exposure	No information is provided to indicate serum HBCD is the appropriate, but the parent compound was measured.	Medium	2	0.200	0.400
	17. Effect biomarker	No biomarker of effect was measured.	Not Rated	NR	NR	NR
	18. Method Sensitivity	Frequency of detection was low. Although they did not provide specific numbers below detection for HBCD, the P75 was still below the LOQ indicating that a large percent was below detection.	Low	3	0.200	0.600
Other	19. Biomarker stability	No information was provided on storage history or stability of the HBCD in the sample.	Medium	2	0.200	0.400
	20. Sample contamination	There is incomplete documentation of the steps taken to provide the necessary assurance that the study data are reliable.	Medium	2	0.200	0.400

Study reference:	Kicinski, M.,Viaene, M. K.,Den Hond, E.,Schoeters, G.,Covaci, A.,Dirtu, A. C.,Nelen, V.,Bruckers, L.,Croes, K.,Sioen, I.,Baeyens, W.,Van Larebeke, N.,Nawrot, T. S. (2012). Neurobehavioral function and low-level exposure to brominated flame retardants in adolescents: A cross-sectional study Environmental Health: A Global Access Science Source, 11(#issue#), 86 HERO ID: 1927571					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Other	21. Method requirements	Solid phase extraction followed by gas chromatography mass spectrometry in electron capture negative ion mode was used. Specifics of the extraction were not provided but are assumed to be the same as used in cited reference (HERO ID 311586). Sensitivity of method for HBCD is not clear as recovery was not reported. The LOQ was 30 ng/L which seems high compared to the other PBDEs and the majority of the samples fell below the LOQ.	Medium	2	0.200	0.400
	22. Matrix adjustment	Don't think matrix adjustment would be appropriate for this biomarker of exposure.	Not Rated	NR	NR	NR
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**6**	**11.5**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.9167**	**Overall Score: Nearest *:**	**1.9**
		Overall Quality Level:		**Medium**		

5.4 Epidemiological evaluation results of the Kicinski et al 2012 study for thyroid outcomes in general

Study reference:	**Kicinski, M.,Viaene, M. K.,Den Hond, E.,Schoeters, G.,Covaci, A.,Dirtu, A. C.,Nelen, V.,Bruckers, L.,Croes, K.,Sioen, I.,Baeyens, W.,Van Larebeke, N.,Nawrot, T. S. (2012). Neurobehavioral function and low-level exposure to brominated flame retardants in adolescents: A cross-sectional study Environmental Health: A Global Access Science Source, 11(#issue#), 86** **HERO ID: 1927571**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Study Participation	1. Participant selection	Participants were recruited during the same time frame (2008-2011) from the same two industrial areas and from the general population of Flemish adolescents using the same criteria. All adolescents from Genk and Menen were eligible. Random sampling of the general population was attained through a multistage sampling design (which is described). Details were provided for all aspects of the selection. The response rates were slightly higher in Genk, but non-responders were noted to not be different from the responders except that there was a higher proportion of girls responding.	High	1	0.400	0.400
	2. Attrition	107 of the 606 subjects included were excluded because of missing covariates (n=84), missing blood measurements (n=3), or did not complete neuro-behavioral tests (n=4). However, results have much fewer numbers for some results without full explanation.	Medium	2	0.400	0.800
	3. Comparison Group	Although a table of characteristics was provided, it was not broken down by area or general population. Differences that were expected to potentially bias the results were included in the analysis. However, there is not enough information provided about the two study areas to determine if there may have been other differences that varied by exposure.	Medium	2	0.200	0.400
Exposure Characterization	4. Measurement of Exposure	HBCD was measured in the serum according to methods by Covaci and Voorspoels (HERO ID 3113586). However, the method they cite does not indicate that this is a method for measuring HBCD nor do they provide recovery rates. Despite that there is no evidence that there would be poor validity or misclassification, it may just be more likely that samples would fall below the LOQ.	Low	3	0.400	1.200
	5. Exposure levels	For HBCD the effects of the concentrations above the LOQ compared to the concentrations below the LOQ were estimated (binary exposure).	Low	3	0.200	0.600
	6. Temporality	The temporality of exposure and outcome is uncertain . The cross-sectional nature of the study design makes it difficult to determine if exposure occurred prior to the outcome.	Low	3	0.400	1.200

Study reference:	Kicinski, M.,Viaene, M. K.,Den Hond, E.,Schoeters, G.,Covaci, A.,Dirtu, A. C.,Nelen, V.,Bruckers, L.,Croes, K.,Sioen, I.,Baeyens, W.,Van Larebeke, N.,Nawrot, T. S. (2012). Neurobehavioral function and low-level exposure to brominated flame retardants in adolescents: A cross-sectional study Environmental Health: A Global Access Science Source, 11(#issue#), 86 HERO ID: 1927571					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	7. Outcome measurement or characterization	Thyroid hormones were measured by competitive immune assays. No other information was provided. These are assumed to be standard assays.	Medium	2	0.670	1.333
	8. Reporting Bias	Information is provided, but not enough for complete extraction (sample size was not specified).	Medium	2	0.330	0.667
Potential Confounding/Variable Control	9. Covariate Adjustment	Gender, age, type of education, parental education, owning the house, smoking , passive smoking, and blood lipids were included in the assessment. BMI, physical activity, computer use, alcohol use, fish consumption, blood lead, serum PCBs were also included in a stepwise regression procedure with p=0.15 for entering and p=0.10 for remaining in the model.	High	1	0.500	0.500
	10. Covariate Characterization	Information was obtained via questionnaires some information to be filled out by the adolescent and some for the parents.	Medium	2	0.250	0.500
	11. Co-exposure Confounding	Two of the groups were selected because they lived near industrial areas. No information was provided on these industrial areas and what else might be there. However, they did account for lead and PCBs (and possibly mercury via fish consumption) because these may impact the results. Although it is unclear if there might be other potential co-exposures, there is no indication that there would be anything additional that would greatly impact the results that was not considered.	Medium	2	0.250	0.500
Analysis	12. Study Design and Methods	The cross-sectional study design is appropriate for evaluating HBCD concentrations in adolescents with thyroid hormone concentrations. The study was part of a biomonitoring program for environmental health surveillance in Flanders, Belgium.	Medium	2	0.400	0.800
	13. Statistical power	Sufficient statistical power with 515 included subjects.	Medium	2	0.200	0.400

Study reference:	**Kicinski, M.,Viaene, M. K.,Den Hond, E.,Schoeters, G.,Covaci, A.,Dirtu, A. C.,Nelen, V.,Bruckers, L.,Croes, K.,Sioen, I.,Baeyens, W.,Van Larebeke, N.,Nawrot, T. S. (2012). Neurobehavioral function and low-level exposure to brominated flame retardants in adolescents: A cross-sectional study Environmental Health: A Global Access Science Source, 11(#issue#), 86** **HERO ID: 1927571**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	14. Reproducibility of analyses	Description is not 100% clear on methods to be reproducible.	Low	3	0.200	0.600
	15. Statistical models	The use of a linear regression or a negative binomial model were acceptable for the data with assumptions met or data transformed.	Medium	2	0.200	0.400
Other	16. Use of Biomarker of Exposure	No information is provided to indicate serum HBCD is the appropriate, but the parent compound was measured.	Medium	2	0.170	0.333
	17. Effect biomarker	Biomarkers of effect shown to have a relationship to health outcomes, but the method is not well validated and the mechanism of action is not understood.	Low	3	0.170	0.500
	18. Method Sensitivity	Frequency of detection of serum HBCD was low. Although they did not provide specific numbers below detection for HBCD, the P75 was still below the LOQ indicating that a large percent was below detection. Sensitivity was likely okay for the thyroid hormones.	Low	3	0.170	0.500
Other	19. Biomarker stability	No information was provided on storage history or stability of the HBCD or thyroid hormones in the sample.	Medium	2	0.170	0.333
	20. Sample contamination	There is incomplete documentation of the steps taken to provide the necessary assurance that the study data are reliable.	Medium	2	0.170	0.333

Study reference:	Kicinski, M.,Viaene, M. K.,Den Hond, E.,Schoeters, G.,Covaci, A.,Dirtu, A. C.,Nelen, V.,Bruckers, L.,Croes, K.,Sioen, I.,Baeyens, W.,Van Larebeke, N.,Nawrot, T. S. (2012). Neurobehavioral function and low-level exposure to brominated flame retardants in adolescents: A cross-sectional study Environmental Health: A Global Access Science Source, 11(#issue#), 86 HERO ID: 1927571					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Other	21. Method requirements	Solid phase extraction followed by gas chromatography mass spectrometry in electron capture negative ion mode was used. Specifics of the extraction were not provided but are assumed to be the same as used in cited reference (HERO ID 311586). Sensitivity of method for HBCD is not clear as recovery was not reported. The LOQ was 30 ng/L which seems high compared to the other PBDEs and the majority of the samples fell below the LOQ. Few details were provided on the thyroid hormone tests.	Medium	2	0.170	0.333
	22. Matrix adjustment	Don't think matrix adjustment would be appropriate for this biomarker of exposure or thyroid hormones.	Not Rated	NR	NR	NR
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**6**	**12.62**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**2.1033**	**Overall Score: Nearest *:**	**2.1**
		Overall Quality Level:		**Medium**		

5.5 Epidemiological evaluation results of the Kim et al 2014 study for thyroid outcomes for mothers & infants

Study reference:	**Kim, U. J.,Oh, J. E. (2014). Tetrabromobisphenol A and hexabromocyclododecane flame retardants in infant-mother paired serum samples, and their relationships with thyroid hormones and environmental factors Environmental Pollution, 184(#issue#), 193-200** **HERO ID: 2324769**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Study Participation	1. Participant selection	Information on participant selection can be found in a related reference—HERO ID 4182288 (Kim et al. 2012). 38 mother-infant pairs agreed to participate and had blood collected at a hospital in Seoul between Nov 2009 and May 2010. Participation eligibility criteria and participation rate were not reported. It is unclear whether this sample was drawn from another previous study (HERO ID 4182289; Kim et al. 2011).	Low	3	0.400	1.200
	2. Attrition	There was no withdrawal of participants from this sample. Use of imputation methods for missing exposure data; exposure measurements (BFR) below the MDL were imputed at 0.5 x MDL to prevent distortion of the data-set, then the data were normalized, excluding outliers, to the total BFR.	High	1	0.400	0.400
	3. Comparison Group	Summary demographic descriptors of the entire population were reported in a prior study (HERO ID 4182288; Kim et al. 2012). Characteristics were not reported by case and control group, but there is no other indication that groups are not similar. It was reported in this reference that controls did not show any symptoms of thyroid disease or other metabolic disorders (including obesity). Therefore, there is indirect evidence (i.e., stated by the authors without providing a description of methods) that cases and controls are similar.	Medium	2	0.200	0.400
Exposure Characterization	4. Measurement of Exposure	HBCD (three diastereomers: alpha-, beta-, gamma-) concentrations were measured in the serum of mothers and infants 1 to 3 months after birth. Quantification methods are provided in Thomsen et al. 2010 [HERO ID 1927695]. HBCDs analyzed by LC/MS/MS. It should be noted that two infants in the case group were unable to have blood drawn in the 1-3 month window. These two infants had samples collected 18-24 months after birth.	High	1	0.400	0.400
	5. Exposure levels	Range is sufficiently large to determine an exposure-response estimate. Ranges were from below MDL (0.05 ng/g lipid) to 91 ng/g lipid. Smallest range was <MDL to 0.991 ng/g lipid. Comparison of means provided a summary measure of exposure levels for each outcome group. For Pearson correlations, the HBCD concentrations were analyzed continuously.	Medium	2	0.200	0.400

Study reference:	Kim, U. J.,Oh, J. E. (2014). Tetrabromobisphenol A and hexabromocyclododecane flame retardants in infant-mother paired serum samples, and their relationships with thyroid hormones and environmental factors Environmental Pollution, 184(#issue#), 193-200 HERO ID: 2324769					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	6. Temporality	Serum samples were taken from mother and infant within the first three months after birth. This does not adequately measure prenatal exposure to HBCDs and serves more as a cross-sectional measure of HBCD concentrations in cases and controls. Serum concentrations from the mother or infant after birth may be related to prenatal exposure but does not give an accurate indication of prenatal exposure and its relationship to congenital hypothyroidism. Thus, the temporality of exposure and outcome is uncertain.	Low	3	0.400	1.200
Outcome Assessment	7. Outcome measurement or characterization	Thyroid hormones were quantified by radioimmunoassay kits (Diagnostic Products Corp., Los Angeles, CA) with a detection limit for T4 and TSH of 1 ug/dL and 0.02 ug/dL, respectively.	High	1	0.670	0.667
	8. Reporting Bias	All of the study's measured outcomes outlined in the abstract, introduction, and methods were discussed in the results. Significant results are presented clearly in tables. However, many non-significant results were discussed in-text only and this does not allow for detailed extraction of non-significant values.	Medium	2	0.330	0.667
Potential Confounding/Variable Control	9. Covariate Adjustment	There is no indication in this reference or the parent reference (HERO ID 4182288; Kim et al. 2012) that potential confounders were considered in the analysis.	Low	3	0.670	2.000
	10. Covariate Characterization	Covariates were not assessed.	Not Rated	NR	NR	NR
	11. Co-exposure Confounding	Other brominated flame retardants were measured and reported in this study. There is no indication of differential exposure between cases and controls.	Medium	2	0.330	0.667
Analysis	12. Study Design and Methods	The study design chosen was appropriate for investigating thyroid hormone levels in relation to exposure to HBCDs. The study uses an appropriate statistical method to address the research question.	Medium	2	0.500	1.000

Study reference:	Kim, U. J.,Oh, J. E. (2014). Tetrabromobisphenol A and hexabromocyclododecane flame retardants in infant-mother paired serum samples, and their relationships with thyroid hormones and environmental factors Environmental Pollution, 184(#issue#), 193-200 HERO ID: 2324769					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	13. Statistical power	The sample size of this study is small. There were 38 mother-infant pairs with only 12 mothers and 12 infants with congenital hypothyroidism (diagnosed in the infant) used in the analysis of correlation between HBCD concentrations and thyroid hormones. It is uncertain if the sample size is adequate to detect an effect in the exposed population.	Medium	2	0.250	0.500
	14. Reproducibility of analyses	The analyses (two-sided student's t-test, normalization of the data set, and outlier exclusions) are presented clearly in the methods and is sufficient to understand precisely what has been done and to be conceptually reproducible with access to the analytic data.	Medium	2	0.250	0.500
	15. Statistical models	No statistical model used.	Not Rated	NR	NR	NR
Other	16. Use of Biomarker of Exposure	Three diastereomers of HBCD were measured in serum, accurately reflecting exposure to HBCDs. These biomarkers are in a specified matrix and are assumed to have an accurate and precise quantitative relationship with exposure.	High	1	0.140	0.143
	17. Effect biomarker	TSH, T4, and other thyroid hormone levels are appropriate measures of thyroid conditions.	High	1	0.140	0.143
	18. Method Sensitivity	The lowest rate of detection for HBCDs was 66% with a MDL of 50 pg/dL. This is low enough to detect chemicals in a sufficient percentage of the samples to address the research question. Analytical methods measuring biomarker are adequately reported.	Medium	2	0.140	0.286
Other	19. Biomarker stability	No apparent issues; storage history is documented.	High	NR	NR	NR

Study reference:	Kim, U. J.,Oh, J. E. (2014). Tetrabromobisphenol A and hexabromocyclododecane flame retardants in infant-mother paired serum samples, and their relationships with thyroid hormones and environmental factors Environmental Pollution, 184(#issue#), 193-200 HERO ID: 2324769					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	20. Sample contamination	Use of blanks and QA/QC documented in detail. Detailed procedures can be found in the supplemental material of a parent reference (HERO ID 4182288; Kim et al. 2012).	High	1	0.140	0.143
Other	21. Method requirements	HBCDs were analyzed by LC/MS/MS (Agilent1200/6460QQQMSD, Agilent Technologies, Santa Clara, CA). Detailed procedures can be found in the supplemental material of a parent reference (HERO ID 4182288; Kim et al. 2012).	High	1	0.140	0.143
	22. Matrix adjustment	HBCDs in serum are presented only as matrix adjusted (ng/g lipid).	Medium	2	0.140	0.286
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**6**	**11.15**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.8583**	**Overall Score: Nearest *:**	**1.9**
		Overall Quality Level:		**Medium**		

5.6 Epidemiological evaluation results of the Meijer et al 2012 study for reproductive outcomes for GIC cohort HBCD sex hormones

Study reference:	**Meijer, L.,Martijn, A.,Melessen, J.,Brouwer, A.,Weiss, J.,de Jong, F. H.,Sauer, P. J. (2012). Influence of prenatal organohalogen levels on infant male sexual development: sex hormone levels, testes volume and penile length Human Reproduction, 27(3), 867-872** **HERO ID: 1401499**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Study Participation	1. Participant selection	Subjects were part of the Groningen-infant-compare cohort (GIC). Cohort consisted of 90 healthy pregnant women, living in the norther provinces of the Netherlands, who delivered a single, term, health infant. This study only focused on the 56 boys born in the cohort; one boy was excluded after ICSI (intracytoplasmic sperm injection) pregnancy, which may predispose to aberrations of sexual development (Wennerholm et al., 2000). How the initial cohort was selected was not determined nor do the study authors provide a citation. However, there is no indication that this sample would not be representative of the exposure-outcome distribution.	Medium	2	0.400	0.800
	2. Attrition	There was minimal subject loss to follow up during the study. One boy was excluded because he was born after ICSI pregnancy, which they indicated could predispose the boy to aberrations of sexual development. HBCD was only measured in 44 of the samples, which were randomly selected, due to financial restraints. Penile length was missing in 8 infants at 18 months due to non-cooperative behavior or loss to follow-up. There is no indication how many of these were from the 44 with measurements for HBCD.	High	1	0.400	0.400
	3. Comparison Group	HBCD was evaluated on a continuous basis and there is no indication that there was anything different about the exposure in this cohort.	Medium	2	0.200	0.400
Exposure Characterization	4. Measurement of Exposure	Maternal serum levels obtained at the 35th week of pregnancy were measured for HBCD levels at the Department of Environmental Chemistry, Stockholm University, Sweden and noted to be described in Meijer et al., 2008 (HERO ID 787696). Cited reference provides complete details including quality control. Therefore, exposure was consistently assessed using well established methods of compound in serum.	High	1	0.400	0.400

Study reference:	Meijer, L.,Martijn, A.,Melessen, J.,Brouwer, A.,Weiss, J.,de Jong, F. H.,Sauer, P. J. (2012). Influence of prenatal organohalogen levels on infant male sexual development: sex hormone levels, testes volume and penile length Human Reproduction, 27(3), 867-872 HERO ID: 1401499					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	5. Exposure levels	Range (not detected to 7.4 ng/g lipid) and distribution (continuous) of exposure is sufficient to establish an exposure response estimate.	Medium	2	0.200	0.400
	6. Temporality	Temporality is established, however, it isn't clear if the levels at 35 weeks of gestation cover the time window relevant to the outcome of interest (male sexual development).	Medium	2	0.400	0.800
Outcome Assessment	7. Outcome measurement or characterization	Testes volume was measured by ultrasound. Measurements were performed by three pediatric radiologists trained for the examination on the same Antares ultrasound machine (Siemens, Erlangen, Germany). Penile length was measured with a standardized tapeline by the same investigator throughout the entire study. A detailed description of how the penile length measurement was made was included. Thus, these outcomes were objectively measured with diagnostic methods and by trained interviewers. There is no reason to believe that the evaluators would be aware of the child's exposure status.	High	1	0.670	0.667
	8. Reporting Bias	All of the study's measured outcomes (primary and secondary) outlined in the methods, abstract, and/or introduction (that are relevant for the evaluation) have not been reported. There are some very general comments for most of the data relevant to the assessment and very little of the HBCD data was actually provided.	Low	3	0.330	1.000
Potential Confounding/Variable Control	9. Covariate Adjustment	No consideration was made for any possible covariates. However, there is no information provided to indicate that there was a significant differential distribution that would have affected the results.	Low	3	0.670	2.000
	10. Covariate Characterization	Covariates were not assessed.	Not Rated	NR	NR	NR

Study reference:	**Meijer, L.,Martijn, A.,Melessen, J.,Brouwer, A.,Weiss, J.,de Jong, F. H.,Sauer, P. J. (2012). Influence of prenatal organohalogen levels on infant male sexual development: sex hormone levels, testes volume and penile length Human Reproduction, 27(3), 867-872** **HERO ID: 1401499**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	11. Co-exposure Confounding	The study measured several OHC compounds in the serum. There is no indication that there is a correlation between any of these compounds. This is a general population study with no reason to believe there would be other differential co-exposures that would affect the results. However, in this cohort, compounds, such as phthalates, that also might be related to sexual development (Hannas et al.,2011) were not analyzed for.	Medium	2	0.330	0.667
Analysis	12. Study Design and Methods	The study design chosen was appropriate for the research question. The study used an appropriate statistical method to address the research question.	Medium	2	0.400	0.800
	13. Statistical power	The number of participants (i.e., 55) seem adequate to detect an effect in the exposed population.	Medium	2	0.200	0.400
	14. Reproducibility of analyses	The description of the analysis is sufficient to understand precisely what was done and to be conceptually reproducible with access to the analytic data.	Medium	2	0.200	0.400
	15. Statistical models	There is a clear description of the analyses.	Medium	2	0.200	0.400
Other	16. Use of Biomarker of Exposure	Maternal serum level of HBCD is the biomarker of exposure and its use is thought to have an accurate and precise quantitative relationship with external exposure.	High	1	0.170	0.167
	17. Effect biomarker	Sex hormones levels are an acceptable biomarker of effect and they were determined at the Endocrine Laboratory, Department of Internal Medicine, Erasmus Medical Centre, Rotterdam, The Netherlands as described elsewhere (Laven et al., 2004).	Medium	2	0.170	0.333

Study reference:	Meijer, L.,Martijn, A.,Melessen, J.,Brouwer, A.,Weiss, J.,de Jong, F. H.,Sauer, P. J. (2012). Influence of prenatal organohalogen levels on infant male sexual development: sex hormone levels, testes volume and penile length Human Reproduction, 27(3), 867-872 HERO ID: 1401499					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	18. Method Sensitivity	Limits of detection are low enough to detect chemicals in a sufficient percentage of the samples to address the research question. Analytical methods measuring biomarker are adequately reported. LOD/LOQ (value or %) are reported. The limit of detection (LOD = three times the standard deviation of the blank values) was 9 pg/g serum for HBCDD. Background levels were subtracted from reported results. HBCDD levels were below LOD in 1/44 samples.	Medium	2	0.170	0.333
Other	19. Biomarker stability	Although the infant serum was stated to be stored at -20 degrees C until analysis, there is no information on how long that was or if there might be any stability issues. No information was provided on the storage or stability of the serum samples for HBCD.	Medium	2	0.170	0.333
	20. Sample contamination	There is incomplete documentation of the steps taken to provide the necessary assurance that the study data are reliable.	Medium	2	0.170	0.333
Other	21. Method requirements	Instrumentation that provides unambiguous identification and quantitation of the biomarker at the required sensitivity (GC–MS) was used.	High	1	0.170	0.167
	22. Matrix adjustment	I don't think this is applicable to either matrix measured.	Not Rated	NR	NR	NR
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**6**	**11.2**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.8667**	**Overall Score: Nearest *:**	**1.9**
		Overall Quality Level:		**Medium**		

5.7 Epidemiological evaluation results of the Meijer et al 2012 study for reproductive outcomes for GIC cohort HBCD male sexual development

Study reference:	**Meijer, L.,Martijn, A.,Melessen, J.,Brouwer, A.,Weiss, J.,de Jong, F. H.,Sauer, P. J. (2012). Influence of prenatal organohalogen levels on infant male sexual development: sex hormone levels, testes volume and penile length Human Reproduction, 27(3), 867-872** **HERO ID: 1401499**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Study Participation	1. Participant selection	Subjects were part of the Groningen-infant-compare cohort (GIC). Cohort consisted of 90 healthy pregnant women, living in the norther provinces of the Netherlands, who delivered a single, term, health infant. This study only focused on the 56 boys born in the cohort; one boy was excluded after ICSI (intracytoplasmic sperm injection) pregnancy, which may predispose to aberrations of sexual development (Wennerholm et al., 2000). How the initial cohort was selected was not determined nor do the study authors provide a citation. However, there is no indication that this sample would not be representative of the exposure-outcome distribution.	Medium	2	0.400	0.800
	2. Attrition	There was minimal subject loss to follow up during the study. One boy was excluded because he was born after ICSI pregnancy, which they indicated could predispose the boy to aberrations of sexual development. HBCD was only measured in 44 of the samples, which were randomly selected, due to financial restraints.	High	1	0.400	0.400
	3. Comparison Group	HBCD was evaluated on a continuous basis and there is no indication that there was anything different about the exposure in this cohort.	Medium	2	0.200	0.400
Exposure Characterization	4. Measurement of Exposure	Maternal serum levels obtained at the 35th week of pregnancy were measured for HBCD levels at the Department of Environmental Chemistry, Stockholm University, Sweden and noted to be described in Meijer et al., 2008 (HERO ID 787696). Cited reference provides complete details including quality control. Therefore, exposure was consistently assessed using well established methods of compound in serum.	High	1	0.400	0.400
	5. Exposure levels	Range (not detected to 7.4 ng/g lipid) and distribution (continuous) of exposure is sufficient to establish an exposure response estimate.	Medium	2	0.200	0.400

Study reference:	Meijer, L.,Martijn, A.,Melessen, J.,Brouwer, A.,Weiss, J.,de Jong, F. H.,Sauer, P. J. (2012). Influence of prenatal organohalogen levels on infant male sexual development: sex hormone levels, testes volume and penile length Human Reproduction, 27(3), 867-872 HERO ID: 1401499					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	6. Temporality	Temporality is established, however, it isn't clear if the levels at 35 weeks of gestation cover the time window relevant to the outcome of interest (male sexual development).	Medium	2	0.400	0.800
Outcome Assessment	7. Outcome measurement or characterization	Sex hormones were measured using acceptable methods and measured at the Endocrine Laboratory, Department of Internal Medicine, Erasmus Medical Centre, Rotterdam, The Netherlands as described elsewhere (Laven et al., 2004). Sex hormones were measured in a specific order due to insufficient amounts of the hormone in some infants.	Medium	2	0.670	1.333
	8. Reporting Bias	All of the study's measured outcomes (primary and secondary) outlined in the methods, abstract, and/or introduction (that are relevant for the evaluation) have not been reported. There are some very general comments for most of the data relevant to the assessment and very little of the HBCD data was actually provided.	Low	3	0.330	1.000
Potential Confounding/Variable Control	9. Covariate Adjustment	No consideration was made for any possible covariates. However, there is no information provided to indicate that there was a significant differential distribution that would have affected the results.	Low	3	0.670	2.000
	10. Covariate Characterization	Covariates were not assessed.	Not Rated	NR	NR	NR
	11. Co-exposure Confounding	The study measured several OHC compounds in the serum. There is no indication that there is a correlation between any of these compounds. This is a general population study with no reason to believe there would be other differential co-exposures that would affect the results. However, in this cohort, compounds, such as phthalates, that also might be related to sexual development (Hannas et al.,2011) were not analyzed for.	Medium	2	0.330	0.667
Analysis	12. Study Design and Methods	The study design chosen was appropriate for the research question. The study used an appropriate statistical method to address the research question.	Medium	2	0.400	0.800

Study reference:	Meijer, L.,Martijn, A.,Melessen, J.,Brouwer, A.,Weiss, J.,de Jong, F. H.,Sauer, P. J. (2012). Influence of prenatal organohalogen levels on infant male sexual development: sex hormone levels, testes volume and penile length Human Reproduction, 27(3), 867-872 HERO ID: 1401499					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	13. Statistical power	The number of participants (i.e., 55) seem adequate to detect an effect in the exposed population.	Medium	2	0.200	0.400
	14. Reproducibility of analyses	The description of the analysis is sufficient to understand precisely what was done and to be conceptually reproducible with access to the analytic data.	Medium	2	0.200	0.400
	15. Statistical models	There is a clear description of the analyses.	Medium	2	0.200	0.400
Other	16. Use of Biomarker of Exposure	Maternal serum level of HBCD is the biomarker of exposure and its use is thought to have an accurate and precise quantitative relationship with external exposure.	High	1	0.170	0.167
	17. Effect biomarker	Sex hormones levels are an acceptable biomarker of effect and they were determined at the Endocrine Laboratory, Department of Internal Medicine, Erasmus Medical Centre, Rotterdam, The Netherlands as described elsewhere (Laven et al., 2004).	Medium	2	0.170	0.333
	18. Method Sensitivity	Limits of detection are low enough to detect chemicals in a sufficient percentage of the samples to address the research question. Analytical methods measuring biomarker are adequately reported. LOD/LOQ (value or %) are reported. The limit of detection (LOD = three times the standard deviation of the blank values) was 9 pg/g serum for HBCDD. Background levels were subtracted from reported results. HBCDD levels were below LOD in 1/44 samples.	Medium	2	0.170	0.333
Other	19. Biomarker stability	Although the infant serum was stated to be stored at -20 degrees C until analysis, there is no information on how long that was or if there might be any stability issues. No information was provided on the storage or stability of the serum samples for HBCD.	Medium	2	0.170	0.333

Study reference:	Meijer, L.,Martijn, A.,Melessen, J.,Brouwer, A.,Weiss, J.,de Jong, F. H.,Sauer, P. J. (2012). Influence of prenatal organohalogen levels on infant male sexual development: sex hormone levels, testes volume and penile length Human Reproduction, 27(3), 867-872 HERO ID: 1401499					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	20. Sample contamination	There is incomplete documentation of the steps taken to provide the necessary assurance that the study data are reliable.	Medium	2	0.170	0.333
Other	21. Method requirements	Instrumentation that provides unambiguous identification and quantitation of the biomarker at the required sensitivity (GC–MS) was used.	High	1	0.170	0.167
	22. Matrix adjustment	I don't think this is applicable to either matrix measured.	Not Rated	NR	NR	NR
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**6**	**11.86**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.9767**	**Overall Score: Nearest *:**	**2**
		Overall Quality Level:		**Medium**		

5.8 Epidemiological evaluation results of the Roze et al 2009 study for neurological/behavior outcomes in general

Study reference:	**Roze, E.,Meijer, L.,Bakker, A.,Van Braeckel, K. N. J. A.,Sauer, P. J. J.,Bos, A. F. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age Environmental Health Perspectives, 117(12), 1953-1958** **HERO ID: 758049**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Study Participation	1. Participant selection	The GIC cohort consisted of 90 white, healthy pregnant women who were randomly selected from those who had given birth to a healthy, full-term, singleton infant. Subjects were selected from the same general population during the same time frame using the same methods. Participation rates and number eligible were not reported. It was noted that all women who had registered with midwives between October 2001 and November 2002 were invited.	Medium	2	0.400	0.800
	2. Attrition	HBCD was only measured in 69 of the 90 women due to financial constraints, but samples were randomly selected. 62 of these actually participated in the follow-up programs. The OHC concentrations of the seven children not followed up were not different from those who did participate. Some results were only available in 57 of the children. Any exclusion of subjects from analyses was adequately addressed and reasons were documented when subjects were removed from the study or excluded from analyses (NTP, 2015a).	High	1	0.400	0.400
	3. Comparison Group	There is only indirect evidence (e.g., stated by the authors without providing a description of methods) that groups are similar with regard to exposure. Some differences in baseline characteristics of groups (such as SES, HOME scores, and sex) were considered as potential confounding and were adjusted for in the analyses.	Medium	2	0.200	0.400
Exposure Characterization	4. Measurement of Exposure	Maternal serum levels obtained at the 35th week of pregnancy were measured for HBCD levels. Noted to be described in Meijer et al., 2008 (HERO ID 787696). Cited reference provides complete details including quality control. Therefore, exposure was consistently assessed using well established methods of compound in the serum.	High	1	0.400	0.400
	5. Exposure levels	Range (0.3-7.5 ng/g lipid) and distribution (continuous) of exposure is sufficient to establish an exposure response estimate.	Medium	2	0.200	0.400
	6. Temporality	Temporality is established. However, it isn't clear if the levels at 35 weeks of gestation cover the time window relevant to the outcome of interest.	Medium	2	0.400	0.800

Study reference:	**Roze, E.,Meijer, L.,Bakker, A.,Van Braeckel, K. N. J. A.,Sauer, P. J. J.,Bos, A. F. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age Environmental Health Perspectives, 117(12), 1953-1958** **HERO ID: 758049**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Outcome Assessment	7. Outcome measurement or characterization	Children were assessed at 5-6 years of age for motor performance, cognition, and behavior. Standardized tests of motor skills for children 4-12 years of age were used for motor outcome. WPPSI-R was used for cognitive outcomes, Touwen's age-specific neurological examination was used to test coordination, balance, and fine manipulative abilities. These are standard methods and are considered to be validated and well-established. The Dutch version of the Developmental Coordination Disorder Questionnaire was also filled out by the parents.	High	1	0.670	0.667
	8. Reporting Bias	All of the study's measured outcomes (primary and secondary) outlined in the methods, abstract, and/or introduction (that are relevant for the evaluation) have not been reported. Although Table 4 provides correlation coefficients for a list of outcomes, it appears that only the significant (less than or equal to a p value of 0.05) or borderline significant effects (less than a p value of 0.10) were reported. For HBCD correlation coefficients were reported for only 3 outcomes.	Low	3	0.330	1.000
Potential Confounding/Variable Control	9. Covariate Adjustment	Results were adjusted for some covariates (such as SES, HOME, and sex) without providing a description of methods.	Medium	2	0.500	1.000
	10. Covariate Characterization	Information was obtained from a questionnaire during the first year after birth. The validity and reliability of this questionnaire was not discussed by the authors.	Medium	2	0.250	0.500
	11. Co-exposure Confounding	The study measured several compounds in the serum. There is no indication that there is a correlation among any of the compounds. This is a general population study with no reason to believe there would be other differential co-exposures that would influence the results.	Medium	2	0.250	0.500
Analysis	12. Study Design and Methods	The prospective cohort study design is appropriate and uses acceptable statistical method (i.e., correlations or Mann-Whitney U test) to address the research question.	Medium	2	0.400	0.800

Study reference:	Roze, E.,Meijer, L.,Bakker, A.,Van Braeckel, K. N. J. A.,Sauer, P. J. J.,Bos, A. F. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age Environmental Health Perspectives, 117(12), 1953-1958 HERO ID: 758049					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	13. Statistical power	The number of participants (i.e., 62) seem adequate to detect an effect in the exposed population.	Medium	2	0.200	0.400
	14. Reproducibility of analyses	The description of the analysis is insufficient to understand what has been done and to be reproducible. Table 4 indicates adjustments for SES, HOME, and sex, but the method description for this was not complete enough to be reproducible.	Low	3	0.200	0.600
	15. Statistical models	As described, it appears that the method is appropriate and that assumptions were met (or data were transformed).	Medium	2	0.200	0.400
Other	16. Use of Biomarker of Exposure	Maternal serum levels of HBCD is a biomarker in a specified matrix that has accurate and precise relationship with external exposure.	High	1	0.200	0.200
	17. Effect biomarker	No biomarker of effect was measured.	Not Rated	NR	NR	NR
	18. Method Sensitivity	Limits of detection are low enough to detect chemicals in a sufficient percentage of the samples to address the research question. Analytical methods measuring biomarkers are adequately reported. LOD/LOQ (value or %) are reported.	Medium	2	0.200	0.400
Other	19. Biomarker stability	No information was provided on storage history or stability.	Low	3	0.200	0.600
	20. Sample contamination	There is incomplete documentation of the steps taken to provide necessary assurance that the study data are reliable.	Medium	2	0.200	0.400

Study reference:	Roze, E.,Meijer, L.,Bakker, A.,Van Braeckel, K. N. J. A.,Sauer, P. J. J.,Bos, A. F. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age Environmental Health Perspectives, 117(12), 1953-1958 HERO ID: 758049					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Other	21. Method requirements	Instrumentation provides unambiguous identification and quantification of the biomarker at the require sensitivity (GC-MS).	Medium	2	0.200	0.400
	22. Matrix adjustment	I don't think any adjustment is needed.	Not Rated	NR	NR	NR
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**6**	**11.07**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.845**	**Overall Score: Nearest *:**	**1.8**
		Overall Quality Level:		**Medium**		

5.9 Epidemiological evaluation results of the Roze et al 2009 study for neurological/behavior outcomes for GIC cohort HBCD neuropsychological

Study reference:	**Roze, E.,Meijer, L.,Bakker, A.,Van Braeckel, K. N. J. A.,Sauer, P. J. J.,Bos, A. F. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age Environmental Health Perspectives, 117(12), 1953-1958** **HERO ID: 758049**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Study Participation	1. Participant selection	The GIC cohort consisted of 90 white, healthy pregnant women who were randomly selected from those who had given birth to a healthy, full-term, singleton infant. Subjects were selected from the same general population during the same time frame using the same methods. Participation rates and number eligible were not reported. It was noted that all women who had registered with midwives between October 2001 and November 2002 were invited.	Medium	2	0.400	0.800
	2. Attrition	HBCD was only measured in 69 of the 90 women due to financial constraints, but samples were randomly selected. 62 of these actually participated in the follow-up programs. The OHC concentrations of the seven children not followed up were not different from those who did participate. Some results were only available in 57 of the children. Any exclusion of subjects from analyses was adequately addressed and reasons were documented when subjects were removed from the study or excluded from analyses (NTP, 2015a).	High	1	0.400	0.400
	3. Comparison Group	There is only indirect evidence (e.g., stated by the authors without providing a description of methods) that groups are similar with regard to exposure. Some differences in baseline characteristics of groups (such as SES, HOME scores, and sex) were considered as potential confounding and were adjusted for in the analyses.	Medium	2	0.200	0.400
Exposure Characterization	4. Measurement of Exposure	Maternal serum levels obtained at the 35th week of pregnancy were measured for HBCD levels. Noted to be described in Meijer et al., 2008 (HERO ID 787696). Cited reference provides complete details including quality control. Therefore, exposure was consistently assessed using well established methods of compound in the serum.	High	1	0.400	0.400
	5. Exposure levels	Range (0.3-7.5 ng/g lipid) and distribution (continuous) of exposure is sufficient to establish an exposure response estimate.	Medium	2	0.200	0.400

Study reference:	**Roze, E.,Meijer, L.,Bakker, A.,Van Braeckel, K. N. J. A.,Sauer, P. J. J.,Bos, A. F. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age Environmental Health Perspectives, 117(12), 1953-1958** **HERO ID: 758049**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	6. Temporality	Temporality is established. However, it isn't clear if the levels at 35 weeks of gestation cover the time window relevant to the outcome of interest.	Medium	2	0.400	0.800
Outcome Assessment	7. Outcome measurement or characterization	Children were assessed at 5-6 years of age for motor performance, cognition, and behavior. Subtests of the NEPSY-II were used to assess neuropsychological function. This is assumed to be a validated standardized test.	High	1	0.670	0.667
	8. Reporting Bias	All of the study's measured outcomes (primary and secondary) outlined in the methods, abstract, and/or introduction (that are relevant for the evaluation) have not been reported. Although Table 4 provides correlation coefficients for a list of outcomes, it appears that only the significant (less than or equal to a p value of 0.05) or borderline significant effects (less than a p value of 0.10) were reported. For HBCD correlation coefficients were reported for only 3 outcomes.	Low	3	0.330	1.000
Potential Confounding/Variable Control	9. Covariate Adjustment	Results were adjusted for some covariates (such as SES, HOME, and sex) without providing a description of methods.	Medium	2	0.500	1.000
	10. Covariate Characterization	Information was obtained from a questionnaire during the first year after birth. The validity and reliability of this questionnaire was not discussed by the authors.	Medium	2	0.250	0.500
	11. Co-exposure Confounding	The study measured several compounds in the serum. There is no indication that there is a correlation among any of the compounds. This is a general population study with no reason to believe there would be other differential co-exposures that would influence the results.	Medium	2	0.250	0.500
Analysis	12. Study Design and Methods	The prospective cohort study design is appropriate and uses acceptable statistical method (i.e., correlations or Mann-Whitney U test) to address the research question.	Medium	2	0.400	0.800

Study reference:	Roze, E.,Meijer, L.,Bakker, A.,Van Braeckel, K. N. J. A.,Sauer, P. J. J.,Bos, A. F. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age Environmental Health Perspectives, 117(12), 1953-1958 HERO ID: 758049					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	13. Statistical power	The number of participants (i.e., 62) seem adequate to detect an effect in the exposed population.	Medium	2	0.200	0.400
	14. Reproducibility of analyses	The description of the analysis is insufficient to understand what has been done and to be reproducible. Table 4 indicates adjustments for SES, HOME, and sex, but the method description for this was not complete enough to be reproducible.	Low	3	0.200	0.600
	15. Statistical models	As described, it appears that the method is appropriate and that assumptions were met (or data were transformed).	Medium	2	0.200	0.400
Other	16. Use of Biomarker of Exposure	Maternal serum levels of HBCD is a biomarker in a specified matrix that has accurate and precise relationship with external exposure.	High	1	0.200	0.200
	17. Effect biomarker	No biomarker of effect was measured.	Not Rated	NR	NR	NR
	18. Method Sensitivity	Limits of detection are low enough to detect chemicals in a sufficient percentage of the samples to address the research question. Analytical methods measuring biomarkers are adequately reported. LOD/LOQ (value or %) are reported.	Medium	2	0.200	0.400
Other	19. Biomarker stability	No information was provided on storage history or stability.	Low	3	0.200	0.600
	20. Sample contamination	There is incomplete documentation of the steps taken to provide necessary assurance that the study data are reliable.	Medium	2	0.200	0.400

Study reference:	Roze, E.,Meijer, L.,Bakker, A.,Van Braeckel, K. N. J. A.,Sauer, P. J. J.,Bos, A. F. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age Environmental Health Perspectives, 117(12), 1953-1958 HERO ID: 758049					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Other	21. Method requirements	Instrumentation provides unambiguous identification and quantification of the biomarker at the require sensitivity (GC-MS).	Medium	2	0.200	0.400
	22. Matrix adjustment	I don't think any adjustment is needed.	Not Rated	NR	NR	NR
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**6**	**11.07**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.845**	**Overall Score: Nearest *:**	**1.8**
		Overall Quality Level:		**Medium**		

5.10 Epidemiological evaluation results of the Roze et al 2009 study for neurological/behavior outcomes for GIC cohort HBCD behavior

Study reference:	**Roze, E.,Meijer, L.,Bakker, A.,Van Braeckel, K. N. J. A.,Sauer, P. J. J.,Bos, A. F. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age Environmental Health Perspectives, 117(12), 1953-1958** **HERO ID: 758049**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Study Participation	1. Participant selection	The GIC cohort consisted of 90 white, healthy pregnant women who were randomly selected from those who had given birth to a healthy, full-term, singleton infant. Subjects were selected from the same general population during the same time frame using the same methods. Participation rates and number eligible were not reported. It was noted that all women who had registered with midwives between October 2001 and November 2002 were invited.	Medium	2	0.400	0.800
	2. Attrition	HBCD was only measured in 69 of the 90 women due to financial constraints, but samples were randomly selected. 62 of these actually participated in the follow-up programs. The OHC concentrations of the seven children not followed up were not different from those who did participate. Some results were only available in 57 of the children. Any exclusion of subjects from analyses was adequately addressed and reasons were documented when subjects were removed from the study or excluded from analyses (NTP, 2015a).	High	1	0.400	0.400
	3. Comparison Group	There is only indirect evidence (e.g., stated by the authors without providing a description of methods) that groups are similar with regard to exposure. Some differences in baseline characteristics of groups (such as SES, HOME scores, and sex) were considered as potential confounding and were adjusted for in the analyses.	Medium	2	0.200	0.400
Exposure Characterization	4. Measurement of Exposure	Maternal serum levels obtained at the 35th week of pregnancy were measured for HBCD levels. Noted to be described in Meijer et al., 2008 (HERO ID 787696). Cited reference provides complete details including quality control. Therefore, exposure was consistently assessed using well established methods of compound in the serum.	High	1	0.400	0.400
	5. Exposure levels	Range (0.3-7.5 ng/g lipid) and distribution (continuous) of exposure is sufficient to establish an exposure response estimate.	Medium	2	0.200	0.400

Study reference:	**Roze, E.,Meijer, L.,Bakker, A.,Van Braeckel, K. N. J. A.,Sauer, P. J. J.,Bos, A. F. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age Environmental Health Perspectives, 117(12), 1953-1958** **HERO ID: 758049**					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	6. Temporality	Temporality is established. However, it isn't clear if the levels at 35 weeks of gestation cover the time window relevant to the outcome of interest.	Medium	2	0.400	0.800
Outcome Assessment	7. Outcome measurement or characterization	Children were assessed at 5-6 years of age for motor performance, cognition, and behavior. To obtain information on the children's competencies and their behavioral and emotional problems, the parents completed the Child behavior checklist and teachers filled out the Teacher's Report Form. Parents also filled out an ADHD questionnaire.	Medium	2	0.670	1.333
	8. Reporting Bias	All of the study's measured outcomes (primary and secondary) outlined in the methods, abstract, and/or introduction (that are relevant for the evaluation) have not been reported. Although Table 4 provides correlation coefficients for a list of outcomes, it appears that only the significant (less than or equal to a p value of 0.05) or borderline significant effects (less than a p value of 0.10) were reported. For HBCD correlation coefficients were reported for only 3 outcomes.	Low	3	0.330	1.000
Potential Confounding/Variable Control	9. Covariate Adjustment	Results were adjusted for some covariates (such as SES, HOME, and sex) without providing a description of methods.	Medium	2	0.500	1.000
	10. Covariate Characterization	Information was obtained from a questionnaire during the first year after birth. The validity and reliability of this questionnaire was not discussed by the authors.	Medium	2	0.250	0.500
	11. Co-exposure Confounding	The study measured several compounds in the serum. There is no indication that there is a correlation among any of the compounds. This is a general population study with no reason to believe there would be other differential co-exposures that would influence the results.	Medium	2	0.250	0.500
Analysis	12. Study Design and Methods	The prospective cohort study design is appropriate and uses acceptable statistical method (i.e., correlations or Mann-Whitney U test) to address the research question.	Medium	2	0.400	0.800

Study reference:	Roze, E.,Meijer, L.,Bakker, A.,Van Braeckel, K. N. J. A.,Sauer, P. J. J.,Bos, A. F. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age Environmental Health Perspectives, 117(12), 1953-1958 HERO ID: 758049					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
	13. Statistical power	The number of participants (i.e., 62) seem adequate to detect an effect in the exposed population.	Medium	2	0.200	0.400
	14. Reproducibility of analyses	The description of the analysis is insufficient to understand what has been done and to be reproducible. Table 4 indicates adjustments for SES, HOME, and sex, but the method description for this was not complete enough to be reproducible.	Low	3	0.200	0.600
	15. Statistical models	As described, it appears that the method is appropriate and that assumptions were met (or data were transformed).	Medium	2	0.200	0.400
Other	16. Use of Biomarker of Exposure	Maternal serum levels of HBCD is a biomarker in a specified matrix that has accurate and precise relationship with external exposure.	High	1	0.200	0.200
	17. Effect biomarker	No biomarker of effect was measured.	Not Rated	NR	NR	NR
	18. Method Sensitivity	Limits of detection are low enough to detect chemicals in a sufficient percentage of the samples to address the research question. Analytical methods measuring biomarkers are adequately reported. LOD/LOQ (value or %) are reported.	Medium	2	0.200	0.400
Other	19. Biomarker stability	No information was provided on storage history or stability.	Low	3	0.200	0.600
	20. Sample contamination	There is incomplete documentation of the steps taken to provide necessary assurance that the study data are reliable.	Medium	2	0.200	0.400

Study reference:	Roze, E.,Meijer, L.,Bakker, A.,Van Braeckel, K. N. J. A.,Sauer, P. J. J.,Bos, A. F. (2009). Prenatal exposure to organohalogens, including brominated flame retardants, influences motor, cognitive, and behavioral performance at school age Environmental Health Perspectives, 117(12), 1953-1958 HERO ID: 758049					
Domain	**Metric**	**Comments**	**Qualitative Determination**	**Metric Score**	**Metric Weighting Factor**	**Weighted Score**
Other	21. Method requirements	Instrumentation provides unambiguous identification and quantification of the biomarker at the require sensitivity (GC-MS).	Medium	2	0.200	0.400
	22. Matrix adjustment	I don't think any adjustment is needed.	Not Rated	NR	NR	NR
High: >=1 and <1.7 Medium: >=1.7 and <2.3 Low: >=2.3 and <=3		**Sum of scores:**			**6**	**11.73**
		Overall Score = Sum of Weighted Scores/Sum of Metric Weighting Factors:		**1.955**	**Overall Score: Nearest *:**	**2**
		Overall Quality Level:		**Medium**		