United States
Environmental Protection Agency

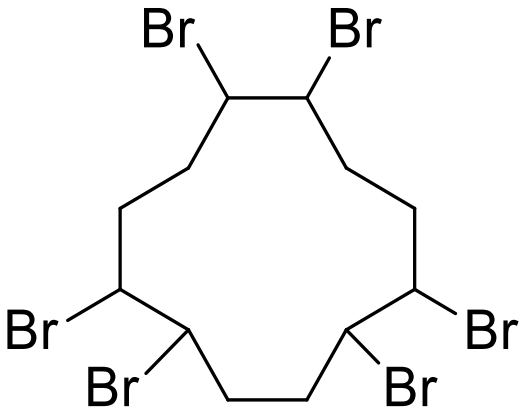
Office of Chemical Safety and
Pollution Prevention

Final 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: 25637-99-4
CASRN: 3194-55-6
CASRN: 3194-57-8



September 2020

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1 Acute (<24 hr)

Table 1: **Animal toxicity evaluation results of Ameribrom Inc 1990 study for primary skin irritation study on irritation outcomes**

Study Citation:	(1990). Letter from Ameribrom Inc to US EPA regarding 8D submission for hexabromocyclododecane with attachments					
Data Type:	Primary skin irritation					
HERO ID:	1928284					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitely and CASRN provided.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported, but the batch/lot number were not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and/or grade of test substance were not reported and there was no report of any analysis conducted for measurement of impurities.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Use of a control group was not reported.
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive controls are required for this kind of study.
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	The study authors did not report how animals were allocated to study groups but there was only one group.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	The authors report that the test substance was used as supplied by the supplier (in carboxymethyl cellulose); however, storage was not reported.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	The study reported consistent exposure administration; however, some details were lacking, such whether the exposures occurred at the same approximate time for all animals.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Administered dose level was reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were reported.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Only one dose level was tested,, but this is acceptable for studies of this type.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was reported and was suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal source, life stage, initial body weight, species, strain, and sex were reported; test animal was from a laboratory-maintained colony

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Study Citation: (1990). Letter from Ameribrom Inc to US EPA regarding 8D submission for hexabromocyclododecane with attachments
Data Type: Primary skin irritation
HERO ID: 1928284

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported, including lighting, temperature, and humidity.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported, appropriate for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcome(s) of interest and was sensitive for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The study authors reported details of the outcome assessment protocol, including time points for post-exposure observations.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	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..
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable for this kind of study.
	Metric 20:	Negative Control Response	Low	× 1	3	The study authors did not report the use of a negative control solvent.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Not Rated	NA	NA	This metric is not applicable.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	There were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	Since most of the endpoints are negative this metric is not applicable.
	Metric 24:	Reporting of Data	Low	× 2	6	There were some deficiencies in reporting of data (e.g., initial body weights were based on a range. rather than actual values.)
Overall Quality Determination‡			Medium		1.8	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i \left(\text{Metric Score}_i \times \text{MWF}_i\right) / \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study.

Table 2: **Animal toxicity evaluation results of Ameribrom Inc 1990 on mortality, body weight outcomes**

Study Citation:	(1990). Letter from Ameribrom Inc to US EPA regarding 8D submission for hexabromocyclododecane with attachments					
Data Type:	Acute oral					
HERO ID:	1928284					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substance was identified.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance, including manufacturer, was not specifically reported. Lot number was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity and grade were not reported and there was no analysis conducted for measurement of impurities, if present.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Use of a control group was not reported, but is not required for studies of this type and outcome
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable since this is a range finding study.
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	This is not applicable since there is only one group.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	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,
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	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.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Administered dose level was reported.
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	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.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Only one dose was tested„ but this is acceptable for studies of this type.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route of exposure was reported and was suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal source, life stage, and starting body weight were not reported; species, strain, and sex were reported.

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Study Citation: (1990). Letter from Ameribrom Inc to US EPA regarding 8D submission for hexabromocyclododecane with attachments
Data Type: Acute oral
HERO ID: 1928284

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	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.
	Metric 15:	Number per Group	High	× 1	1	The number of animals was appropriate for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	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
	Metric 17:	Consistency of Outcome Assessment	Unacceptable	× 1	4	Consistency of the outcome assessments was not adequately reported for meaningful interpretation of results. These are serious flaws that make the study unusable.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding sampling adequacy was not reported and this deficiency is likely to have a substantial impact on results.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not applicable for this study.
	Metric 20:	Negative Control Response	Not Rated	NA	NA	This is not applicable since there is only one group.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	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.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	Not applicable.
	Metric 24:	Reporting of Data	Low	× 2	6	Data reporting was minimal and data on outcomes of exposure were reported in the text only.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		2.4	
Extracted			No			

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Study Citation:	(1990). Letter from Ameribrom Inc to US EPA regarding 8D submission for hexabromocyclododecane with attachments
Data Type:	Acute oral
HERO ID:	1928284

Domain	Metric	Rating†	MWF⋆	Score	Comments††

** Consistent with our *Application of Systematic Review in TSCARisk 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 or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 3: **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 Citation:	IRDC (1978). Acute toxicity studies in rabbits and rats with residue of hexabromocyclododecane with attachments and cover letter dated 030178					
Data Type:	Acute toxicity studies (oral, dermal and occular)					
HERO ID:	787686					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Unacceptable	× 2	8	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; however, it may have been submitted as CBI. The test substance identity and form cannot be determined from the information provided
	Metric 2:	Test Substance Source	Medium	× 1	2	The manufacturer was reported without batch or lot no.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported, but is expected to be low because the 2 samples of the residue had different physical descriptions.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	No vehicle was used for irritation studies. Negative controls are not used for acute toxicity/lethality studies.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not required for irritation or acute toxicity/lethality studies.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage was not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported.

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Study Citation:	IRDC (1978). Acute toxicity studies in rabbits and rats with residue of hexabromocyclododecane with attachments and cover letter dated 030178
Data Type:	Acute toxicity studies (oral, dermal and occular)
HERO ID:	787686

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	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.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Adequate follow up time for examinations for all experiments.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	5 dose groups dermal acute; 6 dose groups oral acute.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain and starting body weight were provided (commercial source, rats and rabbits).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Temperature and humidity controls. Compliance with animal care guidance was indicated.
	Metric 15:	Number per Group	Medium	× 1	2	4-5/sex for oral acute; 2/sex/group for dermal acute; adequate numbers for irritation.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	EPA requestred further information from the TSCA 8e submitter (Velisicol Chemical Corp.) as follows: ""Please describe any gross patholical findings or clinical observation made on the test animals."
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcome(s) of interest were reported and the study used adequate sampling for the outcome(s) of interest.
	Metric 19:	Blinding of Assessors	Low	× 1	3	Information in the study report did not report whether assessors were blinded to treatment group for objective outcomes
	Metric 20:	Negative Control Response	Not Rated	NA	NA	No negative controls
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	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.

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Study Citation:	IRDC (1978). Acute toxicity studies in rabbits and rats with residue of hexabromocyclododecane with attachments and cover letter dated 030178
Data Type:	Acute toxicity studies (oral, dermal and occular)
HERO ID:	787686

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 22: Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Provided references for statistical methods.
	Metric 24: Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group and sex.
Overall Quality Determination‡		Unacceptable⋆⋆		2.0	
Extracted		No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk 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 or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

2 Short-term (1-30 days)

Table 4: **Animal toxicity evaluation results of Maranghi et al 2013 for 28-day dietary study on hepatic, body weight, thyroid, hematological and immune, and reproductive outcomes**

Study Citation:	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
Data Type:	28-day dietary study
HERO ID:	1927558

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Chemical name provided, no CAS #, and no structure provided.
	Metric 2:	Test Substance Source	Low	× 1	3	The source was no reported, no verification or analytical assessment
	Metric 3:	Test Substance Purity	Low	× 1	3	Substance purity was not provided
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	An appropriate negative control was used
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control was not required
	Metric 6:	Randomized Allocation	High	× 1	1	Mice were allocated at random; method used was not detailed
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of exposure diets were described, however the frequency of preparation and details of storage were not indicated.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was consistent across groups. - Animals were restricted to 15% w/w food intake.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	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.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Frequency and duration were clearly reported
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Single dose and a control. - Justification of dose was provided.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route and method was acceptable
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Appropriate test organism

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Study Citation:	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
Data Type:	28-day dietary study
HERO ID:	1927558

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry acceptable
	Metric 15:	Number per Group	High	× 1	1	15/control group 10/treatment group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Methods of outcome assessment were appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling sizes were adequate
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding of assessors was not reported, but is not required for initial histology evaluation.
	Metric 20:	Negative Control Response	High	× 1	1	No abnormal control responses were reported
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were identified.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	There were no unrelated exposure health outcomes
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Appropriate statistical methods were utilized
	Metric 24:	Reporting of Data	High	× 2	2	Data reporting was acceptable
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 5: **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, and hematological and immune outcomes**

Study Citation:	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
Data Type:	28 day feeding study in mice - mechanistic study, animals also infected with RSV
HERO ID:	1927692

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Substance reported as HBCD, no CAS # was provided
	Metric 2:	Test Substance Source	High	× 1	1	Purchased from a commercial source
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported; no validation was done to assess purity
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	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?
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not necessary
	Metric 6:	Randomized Allocation	Low	× 1	3	Randomization was not reported
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Preparation nor storage was reported. Study authors only indicate that HBCD was mixed into a powder diet.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Control and treated Animals were fed ad libitum
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Reported as 1% in diet., body weights and food consumption were provided,
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Daily for 28 days
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	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.
	Metric 12:	Exposure Route and Method	High	× 1	1	Standard exposure route and method
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animals were acceptable
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry was not reported

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Study Citation:	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
Data Type:	28 day feeding study in mice - mechanistic study, animals also infected with RSV
HERO ID:	1927692

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	Study reports use of 6-7 mice/ group; OECD guidelines for 28-day repeated dose study recommends 10 animals/group (5/sex)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	CK: The outcome assessment methodology addressed the intended outcomes
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Methods were acceptable for what they were looking at.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was done on all of the mice/group
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Histology was not done on HBCD treated animals; there were no other subjective outcomes
	Metric 20:	Negative Control Response	High	× 1	1	Control responses were as expected
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no apparently confounding factors that would influence the outcomes
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	There were no unrelated health outcomes
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical method was appropriate for outcome
	Metric 24:	Reporting of Data	High	× 2	2	Reporting of data was accepatble
Overall Quality Determination‡			~~High~~ ⟶ Medium§		~~1.4~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i \left(\text{Metric Score}_i \times \text{MWF}_i\right) / \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "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. This limited endpoints evaluated do not greatly inform mechanism of the potential effects of HBCD on immunity."

Table 6: **Animal toxicity evaluation results of Genskow et al 2015 for 30 day oral toxicity study (daily gavage) with in vitro data on mechanistic and neurological/behavior outcomes**

Study Citation:	Genskow, KR; Bradner, JM; Hossain, MM; Richardson, JR; Caudle, WM (2015). Selective damage to dopaminergic transporters following exposure to the brominated flame retardant, HBCDD Neurotoxicology and Teratology, 52(Pt B), 162-169
Data Type:	30 day oral toxicity study (daily gavage); primarily mechanistic, also contains in vitro data
HERO ID:	2919804

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Test substance name was provided but CAS# was not provided
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance source was provide but batch or lot number was not reported
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substance is not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Vehicle control reported
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control was not necessary, but could have provided useful information in this study that would aid in the interpretation of the results
	Metric 6:	Randomized Allocation	Medium	× 1	2	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.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Details of preparation, frequency of preparation, and storage were lacking
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Control and treatment groups were treated consistently
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Dose concentrations were clearly reported, however, no validation of dose was performed by the study authors.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were clearly reported
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	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.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route and method were acceptable.
Domain 4: Test Organism						

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Study Citation:	Genskow, KR; Bradner, JM; Hossain, MM; Richardson, JR; Caudle, WM (2015). Selective damage to dopaminergic transporters following exposure to the brominated flame retardant, HBCDD Neurotoxicology and Teratology, 52(Pt B), 162-169
Data Type:	30 day oral toxicity study (daily gavage); primarily mechanistic, also contains in vitro data
HERO ID:	2919804

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	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 generally get acclimatized for a week but 4 weeks seem a bit odd.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	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
	Metric 15:	Number per Group	Medium	× 1	2	Four control animals and 6 treated animals of a single sex were used. OECD guidlines for 28-day toxicity studies recommends an n of 10 (5 animals of each sex).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcome(s) of interest and was sensitive for the outcome(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	High	× 1	1	The study reported adequate sampling for the outcome(s) of interest
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not required for this methodology
	Metric 20:	Negative Control Response	High	× 1	1	Control responses appear to be appropriate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	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.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	This information was not included in the study report or in the study design.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was acceptable
	Metric 24:	Reporting of Data	High	× 2	2	Reporting of data (for the methods used) was acceptable.
Overall Quality Determination[‡]			~~High~~ ⟶ Medium[§]		~~1.6~~	
Extracted			Yes			

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Study Citation:	Genskow, KR; Bradner, JM; Hossain, MM; Richardson, JR; Caudle, WM (2015). Selective damage to dopaminergic transporters following exposure to the brominated flame retardant, HBCDD Neurotoxicology and Teratology, 52(Pt B), 162-169
Data Type:	30 day oral toxicity study (daily gavage); primarily mechanistic, also contains in vitro data
HERO ID:	2919804

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "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)"

Table 7: **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, and reproductive outcomes**

Study Citation:	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
Data Type:	acute and 14-day inhalation-systemic tox
HERO ID:	3350482

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was clearly identified by name and CASRN.
	Metric 2:	Test Substance Source	Medium	× 1	2	The test substance source/manufacturer was identified however the batch/lot number was not reported
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was identified
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative control animals were included in the 14 day. No negative control required for acute study.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not applicable.
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly allocated to each group.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The method and equipment used to generate the dust aerosol were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and measured concentrations, MMAD, and GSD were reported for all groups.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Frequency and duration were reported.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of groups and spacing were reported along with rationale for concentration selection.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method were appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The source, health status, species, strain, age, and sex were reported. Initial body weight was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported and appropriate.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was appropriate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported and appropriate.

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Study Citation:	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
Data Type:	acute and 14-day inhalation-systemic tox
HERO ID:	3350482

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling size was adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses were appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables in test design were observed.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported.
Overall Quality Determination‡			High		1.1	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 8: **Animal toxicity evaluation results of Miller et al 2016 for mechanism of liver and thyroid toxicity study on hepatic, thyroid outcomes**

Study Citation:	Miller, I; Serchi, T; Cambier, S; Diepenbroek, C; Renaut, J; Van der Berg, JH; Kwadijk, C; Gutleb, AC; Rijntjes, E; Murk, AJ (2016). Hexabromocyclododecane (HBCD) induced changes in the liver proteome of eu- and hypothyroid female rats Toxicology Letters, 245 40-51
Data Type:	Mechanism of liver and thyroid toxicity
HERO ID:	3350495

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Test substance identified by name. No CAS # or other details were provided
	Metric 2:	Test Substance Source	Low	× 1	3	Source or manufacturer was not identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the substance was not provided
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required.
	Metric 6:	Randomized Allocation	Low	× 1	3	Allocation methods were not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was reported., but storage prior to administration was not reported..
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Appropriate doses were reported
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Frequency and duration were reported.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of groups and spacing were reported
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method were appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	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.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	The temperature, humidity, lighting, water, and diet were reported. No other details were reported.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was appropriate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported and appropriate.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.

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Study Citation:	Miller, I; Serchi, T; Cambier, S; Diepenbroek, C; Renaut, J; Van der Berg, JH; Kwadijk, C; Gutleb, AC; Rijntjes, E; Murk, AJ (2016). Hexabromocyclododecane (HBCD) induced changes in the liver proteome of eu- and hypothyroid female rats Toxicology Letters, 245 40-51
Data Type:	Mechanism of liver and thyroid toxicity
HERO ID:	3350495

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not required.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses were appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Iodine depletion may have an effect on the results
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	One group of animals were exposed in a hypothyroid state.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported.
Overall Quality Determination‡			~~High~~ ⟶ Medium§		~~1.5~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "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 was appropriately conducted."

Table 9: **Animal toxicity evaluation results of Wang et al 2016 for 28 day oral gavage metabolomic study in mice study on nutrition and metabolic/adult exposure body weight, and gene expression/omics outcomes**

Study Citation:	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
Data Type:	28 day oral gavage metabolomic study in mice
HERO ID:	3350496

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified as technical HBCD with 10% alpha, 10% beta, and 80% gamma stereoisomers.
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance obtained from manufacturer but without certification or analytical verification of identity.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity reported as 95%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham-treated controls received vehicle
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for study type
	Metric 6:	Randomized Allocation	High	× 1	1	Study reports random allocation to groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported but storage was not reported
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Time of day of gavage administration was not reported.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Details of exposure administration were reported and exposures were administered consistently across study groups in a scientifically sound manner
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Doses administered daily for 28 days
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 nonzero doses were administered ranging 5-fold. Doses were selected based on reported range of toxic doses
	Metric 12:	Exposure Route and Method	High	× 1	1	oral gavage exposure with appropriate vehicle reported
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animal species, strain, sex, age, and body weight were reported. Females were chosen because they were reportedly more sensitive.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Relative humidity and diet were not reported. All other husbandry conditions were reported and adequate.

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Study Citation:	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
Data Type:	28 day oral gavage metabolomic study in mice
HERO ID:	3350496

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 15: Number per Group	Medium	× 1	2	5 animals/dose tested.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	Medium	× 2	4	Body weight, organ weight and both targeted and untargeted metabolomics were evaluated. BW was measured weekly, but metabolomics only performed once on 24 hr urine samples collected after last dose.
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	No inconsistencies in outcome assessment were noted
	Metric 18: Sampling Adequacy	High	× 1	1	Body weights and metabolomics assessed for individual animals
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	no subjective outcomes
	Metric 20: Negative Control Response	High	× 1	1	Control responses were reported and appeared to be appropriate
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	Medium	× 2	4	Food and water intake were not reported.
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	× 1	2	One control mouse died during the study.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Statistical analysis methods reported and appropriate.
	Metric 24: Reporting of Data	Medium	× 2	4	Body weights reported graphically without measure of variability in supplemental material.
Overall Quality Determination‡		~~High~~ ⟶ Medium§		~~1.4~~	
Extracted		No			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "Although body weight and organ weights were measured, only average body weight was provided in the supplemental material. the author reports organ weight data was not shown, but did not have any changes. This study mainly focus 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."

Table 10: **Animal toxicity evaluation results of Bernhard et al 2016 for 28-day dietary study on hematological and immune, hepatic, and adult body weight outcomes**

Study Citation:	Bernhard, A; Berntssen, MH; Lundebye, A-K; Alvheim, AR; Myrmel, LS; Fjære, E; Torstensen, BE; Kristiansen, K; Madsen, L; Brattelid, T; Rasinger, JD (2016). Marine fatty acids aggravate hepatotoxicity of a-HBCD in juvenile female BALB/c mice Food and Chemical Toxicology, 97 411-423
Data Type:	28-day dietary study
HERO ID:	3545918

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substance was identified definitively and the specific form, however CAS# was not provided
	Metric 2:	Test Substance Source	Low	× 1	3	alpha-HBCD was prepared from gamma-HBCD; however the source of the alpha-HBCD was not reported
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Vehicle (DMSO) dietary control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not needed for repeat dose studies.
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly assigned to groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Although feed and water was changed three times per week and feed intake was recorded, the authors did not indicate how often the diets were freshly prepared. Storage of the test substance was also not provided
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	28-day repeat exposure according to OECD407 guidelines
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Diets were analyzed and daily doses were calculated based on body weights and estimate food intake (15% w/w).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	28-day, continuous exposure.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Dose levels and spacing were justified by the study authors. Selected dose produced a range of responses.
	Metric 12:	Exposure Route and Method	High	× 1	1	Oral - feeding study
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain, sex and starting age were reported (commercial source0.

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Study Citation:	Bernhard, A; Berntssen, MH; Lundebye, A-K; Alvheim, AR; Myrmel, LS; Fjære, E; Torstensen, BE; Kristiansen, K; Madsen, L; Brattelid, T; Rasinger, JD (2016). Marine fatty acids aggravate hepatotoxicity of a-HBCD in juvenile female BALB/c mice Food and Chemical Toxicology, 97 411-423
Data Type:	28-day dietary study
HERO ID:	3545918

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported and appropriate.
	Metric 15:	Number per Group	High	× 1	1	Eight animals per experimental group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Multiple measures of liver effects
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	outcomes were assessed consistently across study groups
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Only 3-4 /group for histopathology and serum chemistry.
	Metric 19:	Blinding of Assessors	Low	× 1	3	Blinding was not reported
	Metric 20:	Negative Control Response	High	× 1	1	Vehicle control was used and appropriate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Food consumption did not differ among groups.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Appropriate and detailed statistical methods were reported
	Metric 24:	Reporting of Data	Medium	× 2	4	Incidence data were not provided for liver histopathology.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 11: **Animal toxicity evaluation results of Bernhard et al 2016 for 28-day oral exposure in mice via diet study on hepatic, and body weight outcomes**

Study Citation:	Bernhard, A; Berntssen, MH; Lundebye, A-K; Alvheim, AR; Myrmel, LS; Fjære, E; Torstensen, BE; Kristiansen, K; Madsen, L; Brattelid, T; Rasinger, JD (2016). Marine fatty acids aggravate hepatotoxicity of a-HBCD in juvenile female BALB/c mice 97 411-423
Data Type:	28-day oral exposure in mice via diet
HERO ID:	3588138

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identity and form are stated, no CAS# reported.
	Metric 2:	Test Substance Source	Medium	× 1	2	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.
	Metric 3:	Test Substance Purity	Low	× 1	3	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.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study used an appropriate vehicle negative control diet.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not necessary
	Metric 6:	Randomized Allocation	Medium	× 1	2	It was stated that animals were randomly assigned, although the method for assignment was not described.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The frequency of diet preparation and a statement about stability were not provided. Preparation of diets was acceptable.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	administration was consistent across groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	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.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Appropriate; study design was based on OECD guideline 407 for short-term repeated dose toxicity study

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Study Citation:	Bernhard, A; Berntssen, MH; Lundebye, A-K; Alvheim, AR; Myrmel, LS; Fjære, E; Torstensen, BE; Kristiansen, K; Madsen, L; Brattelid, T; Rasinger, JD (2016). Marine fatty acids aggravate hepatotoxicity of a-HBCD in juvenile female BALB/c mice 97 411-423
Data Type:	28-day oral exposure in mice via diet
HERO ID:	3588138

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	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)."
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route acceptable
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Standard animal model was used. Age was appropriate for desired "juvenile" developmental time point. Only one sex evaluated. Animals were obtained from Taconic.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry clearly reported and appropriate.
	Metric 15:	Number per Group	Medium	× 1	2	n = 3-8 / group, depending on the outcome evaluated. Sample size is below the recommended minumum (n = 10) for OECD 407.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Methodology of outcome assessments were clearly described and appropriate
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent assessment across groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate. Histology was performed on a subset of animals (n=3-4) from each exposure group, including controls
	Metric 19:	Blinding of Assessors	Medium	× 1	2	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.
	Metric 20:	Negative Control Response	High	× 1	1	No out of the ordinary control responses were noted.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	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
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No health outcomes unrelated to exposure were reported; animals were observed daily.
Domain 7: Data Presentation and Analysis						

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Study Citation:	Bernhard, A; Berntssen, MH; Lundebye, A-K; Alvheim, AR; Myrmel, LS; Fjære, E; Torstensen, BE; Kristiansen, K; Madsen, L; Brattelid, T; Rasinger, JD (2016). Marine fatty acids aggravate hepatotoxicity of a-HBCD in juvenile female BALB/c mice 97 411-423
Data Type:	28-day oral exposure in mice via diet
HERO ID:	3588138

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis methodology were clearly reported and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	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).
Overall Quality Determination‡			~~High~~ ⟶ Medium§		~~1.5~~	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "I would downgrade this study based on concerns related to the purity of the chemical and reporting of the doses/concentrations."

Table 12: **Animal toxicity evaluation results of American Chemistry Council 2003 for short term sensitization study in mice on ear swelling response**

Study Citation:	ACC (American Chemistry Council) (2003). Hexabromocyclododecane: Contact sensitization potential via the local lymph node assay (including a primary irritancy screen) using CBA/J mice
Data Type:	Ear swelling response
HERO ID:	4269880

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitely and the diastereomers information reported.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance reported and adequate to identify its toxicological effects.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors reported using a concurrent negative control group.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control is not necessary for pre-screen irritation test.
	Metric 6:	Randomized Allocation	High	× 1	1	The authors reported randomization of animals based on pre-exposure body weights using a computer program.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	The study authors did not provide details of preparation and storage of the test compound.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Study authors reported details of exposure administration which is uniform across dose groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The exposure doses/concentrations or amounts of test substance were reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure duration and frequency were reported.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose/concentration spacing were justified by study authors.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain and sex were reported. Although starting body weights are not reported, it may not impact the study results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	The study authors report following the OECD 429 guidelines.

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Study Citation:	ACC (American Chemistry Council) (2003). Hexabromocyclododecane: Contact sensitization potential via the local lymph node assay (including a primary irritancy screen) using CBA/J mice
Data Type:	Ear swelling response
HERO ID:	4269880

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	Although the authors used only 1 animal/group, this is appropriate for a pre-screening test for irritation
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Unacceptable	× 2	8	Not a robust test for skin irritation; preliminary test to determine doses for LLNA, evaluates ear swelling only.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	None noted.
	Metric 18:	Sampling Adequacy	High	× 1	1	The authors reported using both the ears of the animal per dose, which is adequate for the ear swelling response.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not a necessary specification in OECD 429 guideline
	Metric 20:	Negative Control Response	High	× 1	1	Ear swelling was minimal for the vehicle control.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were apparent.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Other outcomes not evaluated during the pre-screen.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	Limited sample number precluding ability to do statistical analysis.
	Metric 24:	Reporting of Data	High	× 2	2	Quantitative data provided.
Overall Quality Determination‡			Unacceptable⋆⋆		1.3	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk 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 or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 13: **Animal toxicity evaluation results of American Chemistry Council 2003 for LLN assay for skin sensitization**

Study Citation:	ACC (American Chemistry Council) (2003). Hexabromocyclododecane: Contact sensitization potential via the local lymph node assay (including a primary irritancy screen) using CBA/J mice
Data Type:	LLN assay for skin sensitization
HERO ID:	4269880

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitely and the diastereomers information reported.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance reported and adequate to identify its toxicological effects.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors reported using a concurrent negative control group.
	Metric 5:	Positive Controls	High	× 1	1	Study authors reported using a concurrent positive control group.
	Metric 6:	Randomized Allocation	High	× 1	1	The authors reported randomization of animals based on pre-exposure body weights using a computer program.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	The study authors did not provide details of preparation and storage of the test compound. Study indicates that the test material was not adequately soluble in the preferred vehicle for LLNA assays.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Study authors reported details of exposure administration which is uniform across dose groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The exposure doses/concentrations or amounts of test substance were reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure duration and frequency were reported.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose/concentration spacing were justified by study authors.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain and sex were reported. Although starting body weights are not reported, it may not impact the study results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	The study authors report following the OECD 429 guidelines.

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Study Citation:	ACC (American Chemistry Council) (2003). Hexabromocyclododecane: Contact sensitization potential via the local lymph node assay (including a primary irritancy screen) using CBA/J mice
Data Type:	LLN assay for skin sensitization
HERO ID:	4269880

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of animals per study group was reported which is appropriate for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The study authors followed OECD 429 guidelines.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The study authors did not note any inconsistencies.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcome(s) of interest were reported and the study used adequate sampling for the outcome(s) of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not specified as a requirement in OECD 429 guideline
	Metric 20:	Negative Control Response	Medium	× 1	2	The responses from vehicle control group was higher than other historical controls, but did not alter conclusions of the study.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No significant confounders were identified.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No unrelated heath outcomes.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Appropriate statistical methods utilized.
	Metric 24:	Reporting of Data	High	× 2	2	Quantitative data/results provided.
Overall Quality Determination‡			High		1.1	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 14: **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, and respiratory outcomes**

Study Citation:	WIL Research Laboratories (1997). Twenty-eight day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 3/18/1997					
Data Type:	28-Day Repeated Oral					
HERO ID:	787758					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported, including manufacturer and lot number.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The study authors stated that the purity was "considered to be 100%", but no verification of this purity was reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate concurrent negative control group (administered the vehicle via gavage at the same dose volume).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control is not indicated by study type.
	Metric 6:	Randomized Allocation	Medium	× 1	2	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).
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	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.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	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.

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Study Citation:	WIL Research Laboratories (1997). Twenty-eight day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 3/18/1997
Data Type:	28-Day Repeated Oral
HERO ID:	787758

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	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.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	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.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	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.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure (oral, gavage) were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal source, species, strain, sex, age, and starting body weight (group means) were reported; however, health status was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions (temperature, humidity, light-dark cycle) were reported and were adequate and the same for control and exposed populations.
	Metric 15:	Number per Group	Medium	× 1	2	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).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcomes of interest and was sensitive for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.

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Study Citation:	WIL Research Laboratories (1997). Twenty-eight day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 3/18/1997
Data Type:	28-Day Repeated Oral
HERO ID:	787758

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding the sampling for the outcomes of interest were reported and the study used adequate sampling for the outcomes of interest.
	Metric 19:	Blinding of Assessors	High	× 1	1	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.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control groups were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	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.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	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. "
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for the datasets.
	Metric 24:	Reporting of Data	High	× 2	2	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.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

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Study Citation:	WIL Research Laboratories (1997). Twenty-eight day repeated dose oral toxicity study of HBCD in rats, with cover letter dated 3/18/1997
Data Type:	28-Day Repeated Oral
HERO ID:	787758

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

3 Other

Table 15: **Animal toxicity evaluation results of Miller et al 2016 for 7 day gavage study on proteomic endpoints**

Study Citation:	Miller, I; Renaut, J; Cambier, S; Murk, AJ; Gutleb, AC; Serchi, T (2016). Dataset of liver proteins of eu- and hypothyroid rats affected in abundance by any of three factors: In vivo exposure to hexabromocyclododecane (HBCD), thyroid status, gender differences 8 1344-1347
Data Type:	7 day gavage study of proteomic endpoints
HERO ID:	3546017

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Test substance was identified. The authors have provided two of their previous studies (Miller et al. 2016a,b) for experimental details.
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance source not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The authors have provided two of their previous studies (Miller et al. 2016a,b) for experimental details. So it is assumed that they used concurrent negative controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not typical for this experiment type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The authors have provided two of their previous studies (Miller et al. 2016a,b) for experimental details. But animal allocation was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	The authors have provided two of their previous studies (Miller et al. 2016a,b) for experimental details. But preparation and storage of test substance not reported.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	The authors have provided two of their previous studies (Miller et al. 2016a,b) for experimental details. However, these studies and the present one did not report the details of exposure administration.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	The study authors reported gavage doses but gavage volumes were not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and/or outcome(s) of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose/concentration were reported and spacing were justified.

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Study Citation:	Miller, I; Renaut, J; Cambier, S; Murk, AJ; Gutleb, AC; Serchi, T (2016). Dataset of liver proteins of eu- and hypothyroid rats affected in abundance by any of three factors: In vivo exposure to hexabromocyclododecane (HBCD), thyroid status, gender differences 8 1344-1347
Data Type:	7 day gavage study of proteomic endpoints
HERO ID:	3546017

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test species, strain, and sex were reported. The authors have provided two of their previous studies (Miller et al. 2016a,b) for experimental details.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	The authors have provided two of their previous studies (Miller et al. 2016) for experimental details which included animal husbandry conditions.
	Metric 15:	Number per Group	Medium	× 1	2	The authors have provided two of their previous studies (Miller et al. 2016a,b) for experimental details which included animal husbandry conditions. It is assume that they used 6 rats per group which is not sufficient.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The study authors reported outcome assessment methodology and was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	There is no information available in the publication to determine whether there were inconsistencies in outcome assessment.
	Metric 18:	Sampling Adequacy	Low	× 1	3	The authors have provided two of their previous studies (Miller et al. 2016a,b) for experimental details. However, it is not clear how many animals exposed and/or samples analyzed.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes
	Metric 20:	Negative Control Response	Low	× 1	3	It is not clear from this study how the control responses differed from the test responses.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	It is not clear from this study whether or not there were any confounding variables.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	The study authors haven't provided a discussion on the health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis						

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Study Citation:	Miller, I; Renaut, J; Cambier, S; Murk, AJ; Gutleb, AC; Serchi, T (2016). Dataset of liver proteins of eu- and hypothyroid rats affected in abundance by any of three factors: In vivo exposure to hexabromocyclododecane (HBCD), thyroid status, gender differences 8 1344-1347
Data Type:	7 day gavage study of proteomic endpoints
HERO ID:	3546017

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 23: Statistical Methods	Low	× 1	3	Study authors provided data analysis in supplementary files. Statistical methods were described in their previous paper (Miller et al. 2016a,b). However, they haven't provided the conclusions of their analysis in the present study.
	Metric 24: Reporting of Data	High	× 2	2	The study authors reported data in supplementary material.
Overall Quality Determination‡		~~Medium~~ ⟶ Low§		~~2.0~~	
Extracted		No			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "The data is less amenable for further analysis."

4 Subchronic (30-90 days)

Table 16: **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, and respiratory outcomes**

Study Citation:	WIL Research Laboratories (2001). 90-Day oral (gavage) toxicity study of HBCD in rats					
Data Type:	90-day gavage study					
HERO ID:	787787					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer, lot no. and composite sample nos.
	Metric 3:	Test Substance Purity	High	× 1	1	Composite made from commercial HBCD products. A mix of HBCD, Alpha; HBCD, Beta; HBCD, Gamma; CAS number 3194-55-6. The standards had reported purities of 99.4%,100% and 98.7%. respectively,
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham gavage negative control with corn oil vehicle was used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not used for 90-day studies.
	Metric 6:	Randomized Allocation	High	× 1	1	Computerized randomization.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Stirred until uniform and continuously throughout used. Dosing formulations were prepared weekly.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Dosed daily for 90 days, with 28 day recovery period
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses reported as mg/kg/day, based on most recent bw measurement,
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	90 consecutive days.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	3 treatment groups plus control; not justified by authors, but did produce a range of response
	Metric 12:	Exposure Route and Method	High	× 1	1	Followed OECD Guidelines OECD Guideline 408 and OPPTS 870.3 100 - gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain, sex, age, and starting body weight were reported (commercial source).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported and appropriate.

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Study Citation:	WIL Research Laboratories (2001). 90-Day oral (gavage) toxicity study of HBCD in rats
Data Type:	90-day gavage study
HERO ID:	787787

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	15/sex/group, 1- animals/sex/group used for most outcomes at 13 weeks. FOB was only performed on 5 animals/group. 5 animals/sex/group for 3 weeks and 17 week (recovery) groups for all outcomes and for supplemental analyses (lipid staining).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Thorough outcome assessments.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	High	× 1	1	FOB testing was performed without knowledge of the animal groups assignment. Other outcomes were objective. CK: Functional Observational Battery (FOB) evaluations
	Metric 20:	Negative Control Response	High	× 1	1	Low incidence of histopath. lesions.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	CK: Well described
	Metric 24:	Reporting of Data	High	× 2	2	Summary and indiviual animals tables.
Overall Quality Determination‡			High		1.0	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 17: **Animal toxicity evaluation results of W. I. L. Research 2001 for 90-day gavage study on thyroid outcomes**

Study Citation:	WIL Research Laboratories (2001). 90-Day oral (gavage) toxicity study of HBCD in rats					
Data Type:	90-day gavage study					
HERO ID:	787787					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer, lot no. and composite sample nos.
	Metric 3:	Test Substance Purity	High	× 1	1	Composite made from commercial HBCD products. A mix of HBCD, Alpha; HBCD, Beta; HBCD, Gamma; CAS number 3194-55-6. The standards had reported purities of 99.4%,100% and 98.7%. respectively,
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham gavage negative control with corn oil vehicle was used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not used for 90-day studies.
	Metric 6:	Randomized Allocation	High	× 1	1	Computerized randomization.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Stirred until uniform and continuously throughout used. Dosing formulations were prepared weekly.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Dosed daily for 90 days, with 28 day recovery period
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses reported as mg/kg/day, based on most recent bw measurement,
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	90 consecutive days.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	3 treatment groups plus control; not justified by authors, but did produce a range of response (i.e., thyroid).
	Metric 12:	Exposure Route and Method	High	× 1	1	Followed OECD Guidelines OECD Guideline 408 and OPPTS 870.3 100 - gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain, sex, age, and starting body weight were reported (commercial source).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported and appropriate.

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Study Citation:	WIL Research Laboratories (2001). 90-Day oral (gavage) toxicity study of HBCD in rats
Data Type:	90-day gavage study
HERO ID:	787787

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15:	Number per Group	Low	× 1	3	Only 10/sex/group at each timepoint for organ weights and histopathology. Only 5 animals/sex/group or less (as low as 1) for all TSH controls and week 3/ week 17 measurements (10 for week 13 treatment groups). For T3,T4, 10/sex/group for all week 13 groups (control and treatment) and 5/group for week 3/ week 17.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Thorough outcome assessments.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Objective measurements
	Metric 20:	Negative Control Response	Low	× 1	3	TSH levels in controls were unrealistically low (10-25x below other studies) for the 13-week group in both males and females, with several individual rats below the detection limit. Negative control data was adequate for thyroid weight and pathology.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	
	Metric 24:	Reporting of Data	Low	× 2	6	Summary and individual animal tables are included, however results for thyroid weight differ between summary tables and the text tables in the results section. The text tables show statistically significant increases in females while the summary tables do not.
Overall Quality Determination[‡]			~~High~~ ⟶ Low[§]		~~1.3~~	
Extracted			Yes			

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Study Citation:	WIL Research Laboratories (2001). 90-Day oral (gavage) toxicity study of HBCD in rats
Data Type:	90-day gavage study
HERO ID:	787787

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "While the study is of good quality for other outcomes, the study has several important flaws for interpreting effects on thyroid measurement including: unrealistically low control TSH measurements that were occasionally below the limit of detection, small sample size for thyroid hormone controls, and inconsistent data reporting among tables. The study can contribute to a weight of evidence but is unreliable for use in thyroid dose-response analysis."

Table 18: **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, and thyroid outcomes**

Study Citation:	BASF, (1990). Hexabromocyclododecane 28-day feeding trials with rats with test data and cover letter 900000274 #86-900000274
Data Type:	28-day and 90-day dietary studies
HERO ID:	787638

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by trade name and isomer designation.
	Metric 2:	Test Substance Source	Medium	× 1	2	Source and lot no. were not reported. Manufacturer was assumed to be BASF.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A negative dietary control group was used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not necessary for a 28-day study.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Analysis showed that concentrations remained stable over the week.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Dietary concentrations were not measured analytically, but bw and food consumption were reported for each group.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Diet was administered over 13 weeks (daily was assumed).
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	4 treatment groups plus control; dose response relationships were apparent.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain and starting bw was reported. Not a commercial source, but a laboratory maintained colony.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.
	Metric 15:	Number per Group	High	× 1	1	10/sex/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported.

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Study Citation:	BASF, (1990). Hexabromocyclododecane 28-day feeding trials with rats with test data and cover letter 900000274 #86-900000274
Data Type:	28-day and 90-day dietary studies
HERO ID:	787638

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Data tables are difficult to read, but sampling appears adequate.
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported; however, outcomes were objective.
	Metric 20:	Negative Control Response	Low	× 1	3	Data tables are difficult to read; however, several lesions are noted for controls.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	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.
	Metric 22:	Health Outcomes Unrelated to Exposure	Unacceptable	× 1	4	A large proportion of rats showed signs of respiratory inflammation (47% of controls, 26% of all other rats).
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical analysis was not described clearly, and this deficiency is likely to have a substantial impact on results.
	Metric 24:	Reporting of Data	Low	× 2	6	Data tables are provided for all outcomes by exposure group and sex; however, data are in German and mostly illegible.
Overall Quality Determination‡			Unacceptable⋆⋆		1.8	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk 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 or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 19: **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, and thyroid outcomes**

Study Citation:	ACC (American Chemistry Council) (2002). A 90-day oral (gavage) toxicity study of HBCD in rats
Data Type:	90-day gavage-systemic with sperm evaluations and neurobehavior, same as (2990994)
HERO ID:	4269953

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name, CARSN, structure, molecular formula, and isomer distribution (pp. 1235-1236)
	Metric 2:	Test Substance Source	High	× 1	1	Source and analytical verification were included in the study report.
	Metric 3:	Test Substance Purity	Medium	× 1	2	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.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle control groups were included in the main and satellite studies.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric not applicable.
	Metric 6:	Randomized Allocation	Medium	× 1	2	Animals were allocated by a computerized randomization procedure based on body weight stratification in a block design.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage conditions were reported and appropriate based on stability and homogeneity testing (pp. 1242-1268).
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	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.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Duration of study and frequency of dosing were reported and appropriate for this study
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The selected doses were not justified by study authors, but the doses were adequate to show results relevant to the outcomes of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route and method were suitable.

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Study Citation: ACC (American Chemistry Council) (2002). A 90-day oral (gavage) toxicity study of HBCD in rats
Data Type: 90-day gavage-systemic with sperm evaluations and neurobehavior, same as (2990994)
HERO ID: 4269953

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain, sex, health status, age, and starting body weight were reported. Animals obtained from commercial supplier (Charles River).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Temperature, relative humidity, light/day cycle were reported.
	Metric 15:	Number per Group	High	× 1	1	In general, the number of animals assigned per group was appropriate for the study type and outcome analysis. Group sizes conformed to OECD 408.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	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)
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the protocols used for outcome assessment were reported ad outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling details were well described and adequate.
	Metric 19:	Blinding of Assessors	High	× 1	1	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.

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Study Citation:	ACC (American Chemistry Council) (2002). A 90-day oral (gavage) toxicity study of HBCD in rats
Data Type:	90-day gavage-systemic with sperm evaluations and neurobehavior, same as (2990994)
HERO ID:	4269953

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Negative Control Response	High	× 1	1	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 reponses 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).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No reported differences among the groups were observed.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	There were no health outcomes unrelated to exposure that would influence outcome assessment.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported in tables and in the text for all outcomes.
Overall Quality Determination‡			High		1.1	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

5 Chronic (>90 days)

Table 20: **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, and nutrition and metabolic/adult exposure body weight outcomes**

Study Citation:	Yanagisawa, R; Koike, E; Win-Shwe, TT; 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
Data Type:	14-week study (animals dosed by gavage 1x per week)
HERO ID:	2343717

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Test substance described as HBCD, study did not indicate whether the test substance was composed of different isomers (as other studies have).
	Metric 2:	Test Substance Source	High	× 1	1	Sigma Aldrich - no catalog #
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was not reported, however, products purchased from Sigma for experimental use are generally >95% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	an appropriate vehicle control was used
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control was not necessary
	Metric 6:	Randomized Allocation	High	× 1	1	Mice were randomly allocated. There were no differences in initial BWs
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was described, but the frequency of preparation and storage were not reported.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	All groups appeared to be treated consistently
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dosing was clearly reported, although reported as mg/kg/week CK: Dosing was reported as µg/kg BW/week, not as mg/kg/week
	Metric 10:	Exposure Frequency and Duration	Unacceptable	× 1	4	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.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Three exposure groups and a control.. Justification for exposure levels was provided.

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Study Citation:	Yanagisawa, R; Koike, E; Win-Shwe, TT; 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
Data Type:	14-week study (animals dosed by gavage 1x per week)
HERO ID:	2343717

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	Method of gavage is acceptable, although it is unclear in this case, why a spiked dietary administration wasn't used instead.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Animals, and animal characteristics were all reported, however, only a males were used, for an ~90-day repeated dose study, OECD guideline recommends testing on both sexes
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were appropriate
	Metric 15:	Number per Group	Medium	× 1	2	Only 5-6 animals/group; OECD guidline for 90-day repeated dose study recommends a minimum of 8 animals/group (4 males and 4 females)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Methods used to assess outcomes were appropriate
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	There was consistency across the groups that were tested
	Metric 18:	Sampling Adequacy	Medium	× 1	2	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
	Metric 19:	Blinding of Assessors	High	× 1	1	Study indicates histology was done in a blinded fashion.
	Metric 20:	Negative Control Response	High	× 1	1	No unexpected negative control responses were reported
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were identified.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No unusual health outcomes un-related to the exposure were identified
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was clearly described and appropriate
	Metric 24:	Reporting of Data	Medium	× 2	4	Data presentation was adequate; histological data was presented as images only
Overall Quality Determination‡			Unacceptable⋆⋆		1.4	

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Study Citation:	Yanagisawa, R; Koike, E; Win-Shwe, TT; 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
Data Type:	14-week study (animals dosed by gavage 1x per week)
HERO ID:	2343717

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk 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 or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 21: **Animal toxicity evaluation results of van der Ven 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 Citation:	van der Ven, L.T., Verhoef, A., van de Kuil, T., Slob, W., Leonards, P.E., Visser, T.J., Hamers, T., Herlin, M., Hakansson, 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
Data Type:	280Day Oral Toxicity Study (gavage)
HERO ID:	787745

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	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.
	Metric 2:	Test Substance Source	Medium	× 1	2	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.
	Metric 3:	Test Substance Purity	High	× 1	1	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.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	An appropriate concurrent negative control group was included.
	Metric 5:	Positive Controls	Medium	× 1	2	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.

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Study Citation:	van der Ven, L.T., Verhoef, A., van de Kuil, T., Slob, W., Leonards, P.E., Visser, T.J., Hamers, T., Herlin, M., Hakansson, 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
Data Type:	280Day Oral Toxicity Study (gavage)
HERO ID:	787745

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 6:	Randomized Allocation	Medium	× 1	2	"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.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	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.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and administration was consistent across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Administered doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for this study type and/or outcome(s) of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	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.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain, sex, and age was 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.

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Study Citation:	van der Ven, L.T., Verhoef, A., van de Kuil, T., Slob, W., Leonards, P.E., Visser, T.J., Hamers, T., Herlin, M., Hakansson, 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
Data Type:	280Day Oral Toxicity Study (gavage)
HERO ID:	787745

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Most animal husbandry conditions were reported and adequate. Humidity and temperature was not reported, however, this limitation in reporting is unlikely to have a substantial impact on results.
	Metric 15:	Number per Group	Medium	× 1	2	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'.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology reported and sensitive to the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment methodology were reported and consistent across study groups for the outcomes of interest.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding the sampling for the outcomes of interest were reported and adequate for assessment.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not rated when outcomes are not subjective or for initial histopathology review.
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the negative control group was adequate. As shown in Data tables and in Supplemental tables (ID2919527)
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	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.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	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.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	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.

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Study Citation:	van der Ven, L.T., Verhoef, A., van de Kuil, T., Slob, W., Leonards, P.E., Visser, T.J., Hamers, T., Herlin, M., Hakansson, 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
Data Type:	280Day Oral Toxicity Study (gavage)
HERO ID:	787745

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	High	× 2	2	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).
Overall Quality Determination‡		High		1.3	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

6 Genetic toxicity studies

Table 22: **In vitro evaluation results of Zeiger et al 1987 for Salmonella mutagenicity assay**

Study Citation:	E. Zeiger, B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans, W. Speck (1987). Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals Environmental Mutagenesis, 9(Suppl. 9,Suppl. 9), 1-109
Data Type:	Salmonella mutagenicity assay
HERO ID:	699386

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Reported as "hexabromocyclododecane, mixed isomers"; CASRN 25637-99-4
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Solvent control used (DMSO); author stated that experiments in which the control chemical did not produce a mutagenic response or in which the solvent control values were higher (or lower in the case of TA100 and TA97) than their expected values were rejected.
	Metric 5:	Positive Controls	Medium	× 2	4	Positive controls were run with each trial. Positive control substances are identified by name in the study. The study author notes that experiments were rejected if the positive control did not produce a mutagenic response.
	Metric 6:	Assay Procedures	Medium	× 1	2	Study authors cite another study but also provide a general description of the method.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric does not apply for genotoxicity studies.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Study notes that chemicals known or suspected to be volatile were incubated in capped tubes.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Each experiment followed a consistent protocol.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations reported in Table 123 in Appendix 2.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	48-hour incubation
	Metric 12:	Exposure Route and Method	High	× 1	1	Five concentrations tested; initial testing was done in a toxicity assay to determine the appropriate dose range.

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Study Citation:	E. Zeiger, B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans, W. Speck (1987). Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals Environmental Mutagenesis, 9(Suppl. 9,Suppl. 9), 1-109
Data Type:	Salmonella mutagenicity assay
HERO ID:	699386

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	High	× 1	1	Although the study author cites another source for this method, the study includes enough detail on source, method of preparation and concentration in culture.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test strains described and source was reported.
	Metric 15:	Number per Group	High	× 1	1	Mutagenicity assay tested in triplicate for each strain.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Negative results reported; mean and SEM reported for each test concentration.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment followed a standard protocol.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for mutagenicity studies.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for mutagenicity studies.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	Consistency was maintained across exposure groups.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No information on disproportionate outcomes unrelated to exposure, but this is not expected to impact the study results.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	No statistical analysis, but mean and SEM reported for each group.
	Metric 23:	Data Interpretation	High	× 2	2	Data evaluation protocol described in the text.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Toxicity was evaluated as a decrease in the number of his+ colonies or clearing in the density of the background lawn.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for each exposure group, strain and replicate.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	E. Zeiger, B. Anderson, S. Haworth, T. Lawlor, K. Mortelmans, W. Speck (1987). Salmonella mutagenicity tests: III. Results from the testing of 255 chemicals Environmental Mutagenesis, 9(Suppl. 9,Suppl. 9), 1-109
Data Type:	Salmonella mutagenicity assay
HERO ID:	699386

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 23: **In vitro evaluation results of Ethyl Corporation 1990 for Salmonella/microsomal assay for HBCD**

Study Citation:	Ethyl Corporation (1990). Genetic toxicology salmonella/microsomal assay on hexabromocyclododecane with cover letter dated 030890
Data Type:	Salmonella/microsomal assay for HBCD
HERO ID:	787661

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substance was identified by name and CASRN (3194-55-6) in the submission. In the study itself, the test substance was referred to as "HBCD Bottoms" without additional information.
	Metric 2:	Test Substance Source	Low	× 1	3	The source (manufacturer) of the test substance was not reported; it was not clear if information provided with the test substance (PU-85121 and G.T.# 083) corresponded to batch/lot numbers.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative (untreated) and solvent controls (acetone) were reported; however, data were shown for the solvent control group only.
	Metric 5:	Positive Controls	Medium	× 2	4	Positive controls were included and induced, but were not identified.
	Metric 6:	Assay Procedures	Low	× 1	3	Methods and procedures were described in minimal detail; methods were cited to several company SOPs. The study indicated that OECD requirements were met (presumably for the Ames assay).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Not Rated	NA	NA	No details regarding test substance preparation were reported (cited to company SOPs). The only available information indicates that acetone was used as the solvent for HBCD.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	No details regarding exposure methods were reported (cited to company SOPs).
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	No details regarding exposure duration were reported (cited to company SOPs).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of groups (5 plus controls) and spacing were reported. A rationale for the selection of exposure concentrations was not provided; however, the highest tested dose (5 mg/plate) was in line with recommendations for studies of this type. The exposure concentrations used were considered adequate to evaluate the dose-response.

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Study Citation: Ethyl Corporation (1990). Genetic toxicology salmonella/microsomal assay on hexabromocyclododecane with cover letter dated 030890
Data Type: Salmonella/microsomal assay for HBCD
HERO ID: 787661

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Metabolic Activation	Low	× 1	3	The presence of a metabolic system was reported in the study, but details were not provided (i.e., identification of system used, concentration).
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model (Salmonella typhimurium) was reported. Limited descriptive details were provided, but this test model is routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	Each dose was tested in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	No details regarding outcome assessment methods were reported (cited to company SOPs).
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	No details regarding the consistency of the outcome assessment methods were reported (cited to company SOPs).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial information was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study replicate or group. It was indicated that precipitate (which was observed at all dose levels) interfered with the automatic colony counter at "high dose level;" these levels were counted by hand.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analysis was not conducted and standard deviations were not reported, so independent statistical analysis is not possible. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay.
	Metric 23:	Data Interpretation	Medium	× 2	4	It was inferred from the data table that a 3-fold change compared to the solvent control was considered a positive response.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity analyses are not strictly required by the study type.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination[‡]			Medium		2.0	

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Study Citation:	Ethyl Corporation (1990). Genetic toxicology salmonella/microsomal assay on hexabromocyclododecane with cover letter dated 030890
Data Type:	Salmonella/microsomal assay for HBCD
HERO ID:	787661

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 24: **In vitro evaluation results of Helleday et al 1999 for hprt recombination spd8 and sp5 cells**

Study Citation:	T. Helleday, K. L. Tuominen, A. Bergman, D. Jenssen (1999). Brominated flame retardants induce intragenic recombination in mammalian cells Mutation Research, 439(2,2), 137-147
Data Type:	hprt recombination spd8 and sp5 cells
HERO ID:	787680

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by name and structure.
	Metric 2:	Test Substance Source	Medium	× 1	2	The test substance source manufacturer was reported, but lot or batch was not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative (solvent) controls were reported.
	Metric 5:	Positive Controls	Medium	× 2	4	Concurrent positive control (camptothecin) was reported in text, but results were not reported.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described and were applicable for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was described, but storage conditions were not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration is reported and consistency of administration across groups is inferred from the text
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported clearly.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appropriate for the study type.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure concentrations was reported and appropriate. The spacing was not justified but appeared to be based on growth reduction and colony forming inhibition.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not applicable for this study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Test model was briefly described and previously cited and appeared appropriate for the outcome of interest.

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Study Citation:	T. Helleday, K. L. Tuominen, A. Bergman, D. Jenssen (1999). Brominated flame retardants induce intragenic recombination in mammalian cells Mutation Research, 439(2,2), 137-147
Data Type:	hprt recombination spd8 and sp5 cells
HERO ID:	787680

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of cells was reported and appropriate for each group and each concentration was run in 2 independent experiments.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology was appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to this study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to this study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group or experiment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported, but unlikely to affect the results.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were reported and appropriate for the data.
	Metric 23:	Data Interpretation	High	× 2	2	Criteria were reported and consistent with standards.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity data were reported and methods are commonly used.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and groups.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 25: **In vitro evaluation results of Huntingdon Research Center 1990 for bacterial reverse mutation**

Study Citation:	Huntingdon Research, Center (1990). Ames metabolic activation test to assess the potential mutagenic effect of und no. 49 with cover letter dated 031290					
Data Type:	bacterial reverse mutation assay					
HERO ID:	787683					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	HBCD identified on the cover page with a reference to the full name and CASRN
	Metric 2:	Test Substance Source	Low	× 1	3	No information on source of the test substance provided
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Solvent control included
	Metric 5:	Positive Controls	High	× 2	2	Positive control included
	Metric 6:	Assay Procedures	Low	× 1	3	No information was reported on incubation time or details of bacterial cell growth.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Details were not provided on test substance storage or preparation, although this is less of a concern for a stable powdered compound that would not be expected to easily degrade or vaporize
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	There is no indication that any treatment groups received inconsistent HBCD administration
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Unacceptable	× 2	8	Exposure duration was not reported
	Metric 12:	Exposure Route and Method	High	× 1	1	3 dose groups of 1log spacing is acceptable
	Metric 13:	Metabolic Activation	Medium	× 1	2	Liver microsomal fraction was used for metabolic activation, however no details on concentration or other details were provided
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Strains TA 1535, 1537, 1538, TA 98, and TA 100 are typical and appropriate for an Ames assay but no details were provided and data was not shown for 1537 and 1538.
	Metric 15:	Number per Group	Unacceptable	× 1	4	Number of cells/replicates per group were not reported
Domain 5: Outcome Assessment						

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Study Citation:	Huntingdon Research, Center (1990). Ames metabolic activation test to assess the potential mutagenic effect of und no. 49 with cover letter dated 031290
Data Type:	bacterial reverse mutation assay
HERO ID:	787683

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Assesment addressed the intended outcome of interest
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Details of study protocol execution were not reported
	Metric 18:	Sampling Adequacy	Low	× 2	6	Details on sampling was not reported
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Potential confounders not discussed
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Potential confounders not discussed
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analysis was not described
	Metric 23:	Data Interpretation	Medium	× 2	4	Scoring criteria were not reported but are standard for Ames assays
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity appears to have been performed based on the cover letter but data was not provided
	Metric 25:	Reporting of Data	Medium	× 2	4	Basic revertant number was reported, however only one replication without dose-finding or other details
Overall Quality Determination‡			Unacceptable⋆⋆		2.2	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk 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 or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 26: **In vitro evaluation results of IBT Labs 1990 for in vitro Ames assay in S. typhimurium**

Study Citation:	IBT Labs (1990). Mutagenicity of two lots of FM-100 lot 53 and residue of lot 3322 in the absence and presence of metabolic activation with test data and cover letter 900000267 #86-900000267
Data Type:	in vitro Ames assay in S. typhimurium - HBCD (3194-55-6)
HERO ID:	787688

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The study only refers to formula names of 'Firemaster 100 and residue of FM-100", although both are associated with HBCD and the correct CASRN. Details for the residue form are not provided.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (Great Lakes Chemical Corporation). FM-100 Lot 53
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade of the test substance was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors report using a negative and solvent control (DMSO) for each strain
	Metric 5:	Positive Controls	High	× 2	2	A positive control was tested (N-methyl-N-nitrosoguanidine - MNNG) without metabolic activation and 2-aminofluorene with metabolic activation
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures for the Ames assay were described and was noted to conform to published procedure (Ames et al., 1975)
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was described as diluted in DMSO; storage was not indicated.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were reported to be administered consistently across treated and control groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentration was reported in the results without ambiguity; 25, 50, 100, and 250 ug/10 ml with and without metabolic activation
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	The exposure duration was not reported in the study; however, the test method conformed to the published procedure (Ames et al., 1975)
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure concentrations were reported. The concentrations tested were based on the absence of cytotoxicity. The highest concentration tested was limited by its solubility in dimethylsulfoxide (250 ug/10ul)

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Study Citation:	IBT Labs (1990). Mutagenicity of two lots of FM-100 lot 53 and residue of lot 3322 in the absence and presence of metabolic activation with test data and cover letter 900000267 #86-900000267
Data Type:	in vitro Ames assay in S. typhimurium - HBCD (3194-55-6)
HERO ID:	787688

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	High	× 1	1	S. typhimurium TA-98, TA-100, TA-1535, TA-1537, and TA 1538 were tested with metabolic activation (rat liver microsomes); all but TA 1538 were tested without metabolic activation. The method of preparation and concentrations were reported.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test models were reported and appropriate for the outcome of interest: S. typhimurium TA-98, TA-100, TA-1535, TA-1537, and TA 1538 The source of the bacteria was not reported. Test species were checked for its genetic characteristics in accordance with the Ames protocol.
	Metric 15:	Number per Group	High	× 1	1	The number of organisms were not reported; it was noted that the sample to be tested is 10 ul. 3 replicates per study group were reported. The study was noted to be conducted according to Ames et al., 1975)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was carried out consistently across the controls and treated groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each study replicate or group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported, but this was unlikely to have a substantial effect on results.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were described and appropriate.
	Metric 23:	Data Interpretation	Medium	× 2	4	Tests regarded as mutagenic if the test material is statistically significantly different (significance at the 1% level) from the control and mutations related linearly to dosage.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoints were described (inhibition of growth)

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Study Citation:	IBT Labs (1990). Mutagenicity of two lots of FM-100 lot 53 and residue of lot 3322 in the absence and presence of metabolic activation with test data and cover letter 900000267 #86-900000267
Data Type:	in vitro Ames assay in S. typhimurium - HBCD (3194-55-6)
HERO ID:	787688

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data		× 2	NA	The reporting of data in studies conducted by IBT during 1960-1978 is considered unacceptable due to concerns about the integrity of the lab (i.e., discrepancies between raw data and study report, and gross deficiencies in study conduct were identified during an inspection by the FDA in 1976 and a follow-up audit by EPA and in collaboration with the Canadian Health and Welfare Department). Guidance for review of IBT studies is provided in EPA's Manual for Investigation of HPV Chemicals, based on agreements reached in the OECD Existing Chemicals Programme.
Overall Quality Determination‡		Unacceptable⋆⋆		1.6	
Extracted		No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk 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 or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 27: **In vitro evaluation results of Litton Bionetics 1990 for mutagenicity evaluation**

Study Citation:	Litton Bionetics (1990). Mutagenicity evaluation of 421-32B (final report) with test data and cover letter					
Data Type:	Mutagenicity evaluation for HBCD					
HERO ID:	787698					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substance was identified by name and CASRN (3194-55-6) in the submission. In the study itself, the test substance was referred to as 421-32B or "hexabromocyclododecane dispersion" without additional information.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was not reported; it was not clear if the manufacturer was the submitting organization (Great Lakes Chemical Corporation) cited in the submission.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported. The study indicates that there were no known additives to the test substance(s).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using a concurrent negative (solvent) control group; however, the identity of the solvent was not specified.
	Metric 5:	Positive Controls	High	× 2	2	Concurrent positive controls were used and the intended positive responses were induced. It is noted that a volatile positive control was not used.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were reported in adequate detail (including final concentrations of components in the reaction mixture, initial cell density, temperature).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Preparation of the test substance was reported with missing details (i.e., solvent used).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and applicable to the study type.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (4 plus controls)was reported (slightly lower than 5 recommended number of analyzable concentrations). A rationale for the selection of doses was provided; there was no evidence of toxicity at the lowest dose, and there was evidence of effects at the high dose level.

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Study Citation: Litton Bionetics (1990). Mutagenicity evaluation of 421-32B (final report) with test data and cover letter
Data Type: Mutagenicity evaluation for HBCD
HERO ID: 787698

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Medium	× 1	2	The study authors reported the type, source, and methods of preparation of the metabolic activation system. The concentration of rat liver S9 used in the assays was not specified.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test models (5 strains of Salmonella and Saccharomyces cerevisiae strain D4) were reported and are routinely used for studies of this type. However, the source of these strains (and other descriptive information) was not reported.
	Metric 15:	Number per Group	Low	× 1	3	The number of replicates used were not reported (it is not clear if there was more than 1 plate per dose level). Data were presented as summary data and measured as revertants per plate (therefore, it is possible that multiple plates were used).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported and appropriate for the study type.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analysis was not conducted. not required by study type. and may not have been possible (if only one plate was used per dose). Data were provided with respect to numbers of revertants/plate (without information with respect to sample size, or measure of variance, if applicable).
	Metric 23:	Data Interpretation	High	× 2	2	The study indicates that a dose-related increased number of revertants would be considered a positive response. It is also inferred from the text that a >3-fold increase in the number of revertants could be considered positive (the study states that 2 to 3-fold increases in mutant counts might be within the spontaneous range).

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Study Citation:	Litton Bionetics (1990). Mutagenicity evaluation of 421-32B (final report) with test data and cover letter
Data Type:	Mutagenicity evaluation for HBCD
HERO ID:	787698

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Cytotoxicity Data	Low	× 1	3	The study indicated that cytotoxicity was evaluated, but cytotoxicity and the methods used to determine it were not well-defined. It is stated that toxicity was evaluated at higher doses by assessing background growth. However, it was also indicated that cell survival could not be quantified using plate test procedures (but rather, subjective criteria were applied).
	Metric 25: Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡		Medium		1.7	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 28: **In vitro evaluation results of Microbiological Associates 1996 for CAs in human PBLs**

Study Citation:	Microbiological Associates (1996). Hexabromocyclododecane (HBCD): Chromosome aberrations in human peripheral blood lymphocytes with cover letter dated 12/12/1996					
Data Type:	CAs in human PBLs					
HERO ID:	787699					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by name and CAS 25637-99-4
	Metric 2:	Test Substance Source	Low	× 1	3	The test substance source was not reported. Three industry submitters were identified, but no single source of them test substance was given.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent solvent and cell media controls were reported.
	Metric 5:	Positive Controls	High	× 2	2	Concurrent positive controls were used +/- S9 and had appropriate responses.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were well described and appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage of the test substance was reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Consistency of exposure administration across groups was inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported clearly in tables.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and was longer than guidance values but appears to be appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure groups spacing were justified based on the high dose solubility, number of groups was reported and appropriate.
	Metric 13:	Metabolic Activation	High	× 1	1	Metabolic activation was well described and is commonly used.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model was reported and is commonly used for the outcome of interest.
	Metric 15:	Number per Group	Low	× 1	3	Number per group was not reported, but study was done in duplicate.
Domain 5: Outcome Assessment						

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Study Citation:	Microbiological Associates (1996). Hexabromocyclododecane (HBCD): Chromosome aberrations in human peripheral blood lymphocytes with cover letter dated 12/12/1996
Data Type:	CAs in human PBLs
HERO ID:	787699

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was carried out consistently across groups.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	Number of cells samples is 100/replicate and is less than recommended.
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding of assessors was reported.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each group and replicate.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported, but this is unlikely to have a substantial impact on results.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was reported and was appropriate for the study type.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria were reported and consistent with guideline.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity was evaluated based on mitotic inhibition and is commonly used.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all groups and outcomes.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 29: **In vitro evaluation results of Pharmakologisches Institut 1990 for Ames test**

Study Citation:	Pharmakologisches Institut (1990). Ames test with hexabromides with cover letter dated 031290					
Data Type:	Ames test for HBCD					
HERO ID:	787701					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by chemical name, CASRN (3194-55-6), and structure in the submission. In the study itself, the test substance was referred to as "Hexabromid S" without additional information.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was not reported (unclear if the source was the submitting organization).
	Metric 3:	Test Substance Purity	High	× 1	1	The reported purity of the test substance (approximately 95%) is such that effects likely due to test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative (DMSO-only) controls were included; all conditions except exposure to the test substance appeared to be equal.
	Metric 5:	Positive Controls	High	× 2	2	Concurrent positive controls were used, and the intended positive response was induced.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures (namely modifications) were briefly reported; methods were partially cited to another publication.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The study indicates that the test substance was stored at 4C in the dark and dissolved in the solvent on the day of the mutagenicity experiment.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appropriate for the study type.

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Study Citation: Pharmakologisches Institut (1990). Ames test with hexabromides with cover letter dated 031290
Data Type: Ames test for HBCD
HERO ID: 787701

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups was reported (5 plus controls in the absence of activation and 7 plus controls in the presence of activation). The number of exposure groups aligns with the number recommended for studies of this type; however, no rationale for dose selection (other than indications of precipitation at 1000 ug/plate) was provided.
	Metric 13:	Metabolic Activation	High	× 1	1	The study authors reported exposures were conducted in the presence of metabolic activation and the type and source, method of preparation,and volume in final culture of the metabolic activation system were described.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test models (Salmonella strains) were described in detail in the Introduction of the study and are routinely for the outcome of interest. The source of the bacterial strains was not specified.
	Metric 15:	Number per Group	High	× 1	1	The number of replicates (two plates) was reported and appropriate for the study type. It is noted that one plate was available for the 315 ug/plate dose with activation; no explanation was provided.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported and appropriate for the study type.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	High	× 1	1	The study indicated that the person that counted colonies did not know the specifications of the plates.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	The test material interfered in the assay (i.e., precipitation at 1000 ug/plate and above). The study authors indicated that this was not expected to be a study limitation, since mutagenicity is expected at lower doses.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Data were not analyzed statistically, but were raw data were presented so that analyses could be conducted independently.

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Study Citation:	Pharmakologisches Institut (1990). Ames test with hexabromides with cover letter dated 031290
Data Type:	Ames test for HBCD
HERO ID:	787701

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	Medium	× 2	4	The criteria for a positive response was not clearly specified; however, this omission is not expected to substantially impact the study results.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity was not included in the study and are not strictly required by study type.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 30: **In vitro evaluation results of SRI International 1990 for mutagenicity studies**

Study Citation:	SRI International (1990). In vitro microbiological mutagenicity studies of four Ciba-Geigy Corporation compounds (final report) with test data and cover letter
Data Type:	Mutagenicity studies for HBCD
HERO ID:	787716

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substance was identified by name and CASRN (3146-5-6) in the submission. In the study itself, the test substance was referred to as 421-3B "hexabromocyclododecane dispersion" without further information.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was not reported; it was not clear if the source was the submitting organization (Great Lakes Chemical Corporation) or corporation for which the report was prepared (as this test substance and others were called "Ciby-Geigy Corporation compounds").
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported. the study indicated that there were no known additives to the test substance(s).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study reported using concurrent negative (solvent only) controls.
	Metric 5:	Positive Controls	Medium	× 2	4	Concurrent positive controls were used and the intended positive response was induced. The study indicated that each culture was tested using specific mutagens (positive controls); however, it appears from the data tables that only two of five strains were tested with activation (all were tested without activation).
	Metric 6:	Assay Procedures	High	× 1	1	Methods and procedures were adequately described (including reaction mix, temperature, and media).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The study indicated that the test substance was dissolved in the solvent immediately before use. Storage conditions were not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.

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Study Citation:	SRI International (1990). In vitro microbiological mutagenicity studies of four Ciba-Geigy Corporation compounds (final report) with test data and cover letter
Data Type:	Mutagenicity studies for HBCD
HERO ID:	787716

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and adequate for the study type.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups (7 plus controls) was reported. A rationale for the selection of dose groups was not provided other than a statement indicating that the test substance was tested over a wide range of doses for toxicity and mutagenicity determinations.
	Metric 13:	Metabolic Activation	High	× 1	1	The study authors reported exposures were conducted in the presence of metabolic activation and the type and source, method of preparation, and volume in final culture of the metabolic activation system were described.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (Salmonella typhimurium strains) and descriptiove information were reported. The source of the model was a laboratory-maintained culture, and this test model is routinely used for assays of this type.
	Metric 15:	Number per Group	Medium	× 1	2	The study indicated that results were the average of at least two experiments (conducted on two separate days). The results are presented as the average number of revertants per plate, indicating that there were multiple plates per exposure group (number not explicitly specified).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome methodology assessment was reported and appropriate for the study type.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	No data were reported for outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis						

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Study Citation:	SRI International (1990). In vitro microbiological mutagenicity studies of four Ciba-Geigy Corporation compounds (final report) with test data and cover letter
Data Type:	Mutagenicity studies for HBCD
HERO ID:	787716

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analysis was not conducted and standard deviations were not reported, so independent statistical analysis is not possible. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay.
	Metric 23:	Data Interpretation	Low	× 2	6	The criteria for a positive response was not explicitly specified.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity analyses are not strictly required by study type.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡			High		1.6	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 31: **In vitro evaluation results of An et al 2013 for Comet assay on L02 cell line**

Study Citation:	J. An, W. Zou, C. Chen, F. Y. Zhong, Q. Z. Yu, Q. J. Wang (2013). The cytological effects of HBCDs on human hepatocyte L02 and the potential molecular mechanism Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering, 48(11,11), 1333-1342
Data Type:	Comet assay on L02 cell line
HERO ID:	1927550

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as hexabromocyclodecanes.
	Metric 2:	Test Substance Source	High	× 1	1	Test substance was purchased from TCI (Tokyo, Japan).
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity or grade of test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Concurrent negative controls were included in assays; however, it is not clear whether the controls were treated with DMSO (media or solvent/vehicle controls).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not used, but may not have been needed in the mechanistic context of the study. A clear dose-response relationship was demonstrated for DNA damage.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods were well described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of test substance was reported; however, storage conditions, or if substance was made immediately prior to use, were not given.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration were reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups were appropriate to establish dose-response relationships.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	It was assumed that the human hepatocyte cell line does not require exogenous metabolic activation.
Domain 4: Test Model						

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Study Citation:	J. An, W. Zou, C. Chen, F. Y. Zhong, Q. Z. Yu, Q. J. Wang (2013). The cytological effects of HBCDs on human hepatocyte L02 and the potential molecular mechanism Journal of Environmental Science and Health, Part A: Toxic/Hazardous Substances & Environmental Engineering, 48(11,11), 1333-1342
Data Type:	Comet assay on L02 cell line
HERO ID:	1927550

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	Test model was identified as an immortalized human hepatocyte (L02) cell line obtained from Ping Zhou (Beijing Institute of Radiation Medicine, Beijing China).
	Metric 15:	Number per Group	High	× 1	1	All experiments were performed in at least triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported and is sensitive for the outcome or interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessments were carried out consistently.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate for the outcome of interest (100 cells/sample).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Automated measurements were made using fluorescence analysis.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported by group or replicate.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	ANOVA and a post hoc Tukey test were performed.
	Metric 23:	Data Interpretation	High	× 2	2	Data scoring and evaluations were appropriate.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxic endpoints were clearly defined.
	Metric 25:	Reporting of Data	High	× 2	2	Data were presented clearly in figures and in the text.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 32: **In vitro evaluation results of Ethyl Corporation 1990 for DNA repair in rat hepatocytes**

Study Citation:	Ethyl Corporation (1990). Genetic toxicology rat hepatocyte primary culture/DNA repair test on hexabromocyclododecane with cover letter dated 030890
Data Type:	DNA repair in rat hepatocytes for HBCD
HERO ID:	1928253

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substance was identified by name and CASRN (3194-55-6) in the submission. In the study itself, the test substance was referred to as "HBCD Bottoms" without additional information.
	Metric 2:	Test Substance Source	Low	× 1	3	The source (manufacturer) of the test substance was not reported; it was not clear if information provided with the test substance (PU-85121 or G.T.# 083) corresponded to batch/lot numbers.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative (untreated) and solvent controls (acetone) were reported; however, data were shown for the solvent control group only. The response of the vehicle-only controls was acceptable.
	Metric 5:	Positive Controls	High	× 2	2	A concurrent positive control group was used (2-AAF) and the intended positive response was induced.
	Metric 6:	Assay Procedures	Low	× 1	3	Methods and procedures were described in minimal detail; methods were cited primarily to company SOPs. An SOP entitled "Rat Hepatocyte Primary Culture/DNA Repair Test" was included with the study (but is not specific to the study).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The study indicates that the color and appearance of the test substance did not change from the time of receipt until the time of use. Acetone was identified as the solvent used in the experiment. The company SOP provided with the study (but not specific to the study) indicated that solvent selection is based on the solubility and/or suspendability of the test substance. There was no information on storage conditions.

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Study Citation:	Ethyl Corporation (1990). Genetic toxicology rat hepatocyte primary culture/DNA repair test on hexabromocyclododecane with cover letter dated 030890
Data Type:	DNA repair in rat hepatocytes for HBCD
HERO ID:	1928253

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Limited data were provided; however, the study indicated that application volumes were consistent (i.e., 20 uL HBCD bottoms added to 2 mL of media). It is inferred from the text that exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	The exposure duration (and details) were cited to a company SOP (ETTOX 029).
	Metric 12:	Exposure Route and Method	High	× 1	1	The study used 10 concentrations plus controls; cytotoxicity was reported at the highest dose. The SOP provided for the study type (but not specific to the study) indicated that highest doses would be 5% or the highest soluble concentration and remaining doses would be half-log dilutions.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model (rat hepatocytes) was reported with limited descriptive information. This test model is routinely used to evaluate the outcome of interest. The SOP provided with the study (but not specific to the study) indicated that rat hepatocytes are used in this assay because they have been shown to incorporate 3H-thymidine into DNA due to unscheduled DNA synthesis. Some details regarding the test model were cited to other company SOPs.
	Metric 15:	Number per Group	Not Rated	NA	NA	The number of replicates was not reported; detailed methods were cited to company SOPs.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methods were partially described (UDS as measured as a net nuclear increase in grain count) and partially cited to company SOPs.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Outcome assessment methods were cited to company SOPs, but some details were partially described and consistency of evaluations appeared appropriate.

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Study Citation:	Ethyl Corporation (1990). Genetic toxicology rat hepatocyte primary culture/DNA repair test on hexabromocyclododecane with cover letter dated 030890
Data Type:	DNA repair in rat hepatocytes for HBCD
HERO ID:	1928253

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 18:	Sampling Adequacy	High	× 2	2	The SOP provided with the study (but not specific to the study) stated that nuclear and background grain counts should be quantified in 25 cells/slide or as many cells as possible per slide (up to 25) in the presence of cytotoxicity. The data table indicates that 25 cells were counted on one slide (unclear which group(s) this refers to).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions for each study group were not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	Statistical methods were reported with omissions. The study stated that a Chi square analysis was done to compare treated cells to untreated cells, and that statistical significance was achieved at the top 4 concentrations (not indicated as positive in the accompanying data table).
	Metric 23:	Data Interpretation	High	× 2	2	The criteria for a positive response was explicitly specified. The study indicated that the result for HBCD was considered positive because it produced a mean grain count of 5 or greater than the negative control mean grain count and a statistically significant change between HBCD-treated cells and the controls in the number of cells with grain counts > 0.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	It is reported that cytotoxicity was assessed (as cytotoxicity was noted at 1000 ug/well); however, the endpoint was not well-defined and the methods of measurement were not reported.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination[‡]			Medium		1.8	
Extracted			Yes			

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Study Citation:	Ethyl Corporation (1990). Genetic toxicology rat hepatocyte primary culture/DNA repair test on hexabromocyclododecane with cover letter dated 030890
Data Type:	DNA repair in rat hepatocytes for HBCD
HERO ID:	1928253

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 33: **In vitro evaluation results of Ameribrom Inc 1990 for bacterial reverse mutation**

Study Citation:	(1990). Letter from Ameribrom Inc to US EPA regarding 8D submission for hexabromocyclododecane with attachments					
Data Type:	Bacterial reverse mutation					
HERO ID:	1928284					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name, chemical formula, and physical chemical properties.
	Metric 2:	Test Substance Source	Medium	× 1	2	Source was identified as Bromine Compounds Ltd; batch/lot number was not given.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative controls were included.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were included.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Criteria not required.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Preparation details were described.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Duration was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of groups and spacing were reported with justification.
	Metric 13:	Metabolic Activation	High	× 1	1	Activation system and mix were described.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test models were well described.
	Metric 15:	Number per Group	Medium	× 1	2	An overnight culture was used for experiments, but exact number of cells not reported. The number of replicates was reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was described.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not required.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each study replicate or group.

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Study Citation:	(1990). Letter from Ameribrom Inc to US EPA regarding 8D submission for hexabromocyclododecane with attachments
Data Type:	Bacterial reverse mutation
HERO ID:	1928284

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Sterility check plates were used to show that the test material and S9 mix were free of microbial contamination.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were described.
	Metric 23:	Data Interpretation	High	× 2	2	Criteria for positive finding was described.
	Metric 24:	Cytotoxicity Data	High	× 1	1	A preliminary cytotoxicity assay was conducted.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 34: **In vitro evaluation results of GSRI 1978 for Ames assay in S. typhimurium**

Study Citation:	GSRI (1978). Mutagenicity test of GLS-S6-41A (not published)					
Data Type:	in vitro mutation Ames assay in S. typhimurium					
HERO ID:	1937197					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Low	× 2	6	The test substance was identified as GLS-S6-41A in the study report. A hand-written notation on the cover page of the study identifies this substance as hexabromocyclododecane; specific form was not reported.
	Metric 2:	Test Substance Source	Low	× 1	3	The source of the test substance was not reported. A hand-written notation on the cover page of the study identifies Ethyl Corp., but it is not clear if this is the source of the test substance.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade of the test substance was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study results report using a solvent control (DMSO 0.1 ml) for each strain
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were tested (Benzo(a)pyrene (TA98 and TA100, N-methyl-N-nitroso N-nitroguanidine (TA1535), 9-aminoacridine (TA 1537)
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay methods and procedures for the Ames assay were not described, but was noted to conform to published procedure (Ames et al., 1975)
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was not described but can be assumed to be prepared by dilution in DMSO as this is noted to be the solvent control.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposures can be inferred to be administered consistently across treated and control groups; application methods were not described.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentration was reported in the results without ambiguity; 2, 40, 200, and 1000 ug/plate with and without metabolic activation
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	The exposure duration was not reported in the study; however, the test method was noted to be conducted according to the published procedure (Ames et al., 1975)

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Study Citation: GSRI (1978). Mutagenicity test of GLS-S6-41A (not published)
Data Type: in vitro mutation Ames assay in S. typhimurium
HERO ID: 1937197

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure concentrations and spacing were reported in the results. the number of exposure groups and spacing of exposure levels appear to be adequate to show results relevant to the outcome of interest.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The presence of a metabolic activation system was reported in the study. Details regarding type, composition mix, concentration, or quality control information were not described. The activity of the liver homogenate was noted to be validated by its ability to convert the positive controls to mutagenic products.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was reported but no additional details were reported: S. typhimurium TA-98, TA-100, TA-1535, TA-1537. The source of the bacteria was not reported.
	Metric 15:	Number per Group	Unacceptable	× 1	4	The number of organisms or tissues per study group and replicates per study group were not reported
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology used only partially addressed or reported the intended outcomes of interest (mutation frequency evaluated in the absence of cytotoxicity in a gene mutation test).
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There was incomplete reporting of minor details of outcome assessment protocol execution; however, the study was noted to be conducted according to the published protocol (Ames et al. 1975)
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This method is not applicable to the outcome.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no confounding variables noted in the study
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	S. typhimurium TA100 and TA98 were noted to have intrinsically high spontaneous mutation frequency, and therefore comparison to background levels should be interpreted cautiously. It appears there were no replicates to the assay.
Domain 7: Data Presentation and Analysis						

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Study Citation:	GSRI (1978). Mutagenicity test of GLS-S6-41A (not published)
Data Type:	in vitro mutation Ames assay in S. typhimurium
HERO ID:	1937197

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Low	× 1	3	Statistics were not used to assess increased revertants/plate, either from control or comparing with/without metabolic activation. A positive result was not specifically defined, but it was suggested that it was related to increased revertants and dose-dependency. Statistical analysis is not necessarily required for the bacterial reverse mutation assay, so the data analysis is considered acceptable."
	Metric 23:	Data Interpretation	Low	× 2	6	Scoring and/or evaluation criteria were not reported
	Metric 24:	Cytotoxicity Data	Unacceptable	× 1	4	Cytotoxicity endpoints were not defined, methods were not described, and it could not be determined that cytotoxicity was accounted for in the interpretation of study results
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcome were presented for each study group.
Overall Quality Determination‡			Unacceptable**		2.1	
Extracted			No			

** Consistent with our *Application of Systematic Review in TSCARisk 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 or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 35: **In vitro evaluation results of An et al 2016 for Comet assay**

Study Citation:	An, J; Guo, P; Shang, Y; Zhong, Y; Zhang, X; Yu, Y; Yu, Z (2016). The "adaptive responses" of low concentrations of HBCD in L02 cells and the underlying molecular mechanisms Chemosphere, 145 68-76
Data Type:	Comet assay for HBCD
HERO ID:	3350502

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance (a manufacturer) was reported; no information on a batch/lot number was provided.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity of the test substance was not reported. The study indicates that HBCD was from a manufacturer and "all other chemicals were analytical reagents."
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study used concurrent negative (vehicle-only) control groups. The study evaluated DNA breaks after low +/- high exposures to HBCD; appropriate control groups were used (i.e., no treatment, only high-dose treatment).
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type. However, test substances used in the assay showed positive dose-related responses.
	Metric 6:	Assay Procedures	High	× 1	1	Comet assay procedures were described in adequate detail (e.g., cell density, volumes, temperature).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	information related to the preparation, storage, and stability of the test substance (in solvent) were not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures appeared to be administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The duration of exposures were reported. The typical duration of exposure for an in vitro comet assay is 3 to 6 hours. This study evaluated the low dose HBCD exposures +/- subsequent high-dose HBCD exposure; in each case, exposures were 48 hours (presumably based on earlier studies).

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Study Citation:	An, J; Guo, P; Shang, Y; Zhong, Y; Zhang, X; Yu, Y; Yu, Z (2016). The "adaptive responses" of low concentrations of HBCD in L02 cells and the underlying molecular mechanisms Chemosphere, 145 68-76
Data Type:	Comet assay for HBCD
HERO ID:	3350502

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of exposure groups was reported. Two HBCD low exposure conditions were used (10ˆ-13 and 10ˆ-11 M; three are recommended), and one high dose of HBCD (50 uM) was used thereafter. The low doses were selected because they were considered environmentally-relevant. A rationale for the high dose was not provided;, and the high dose induced significant toxicity (survival as low as 60% of controls).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was described with limited details, and the source was not reported. The study indicated that the cell line was selected because the liver is a primary target of xenobiotics, and the cell line is routinely used to investigate cell signaling pathways (the focus of the study).
	Metric 15:	Number per Group	High	× 1	1	The study indicated that each experiment was performed in triplicate using three replicates per sample.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables in test design were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No outcomes unrelated to exposure were reported (and are not expected to impact the study results).
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were reported and appropriate for the study type.
	Metric 23:	Data Interpretation	Medium	× 2	4	Statistical significance appears to have been the criteria for a positive response (recommended as an aid in determining positive responses).

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Study Citation:	An, J; Guo, P; Shang, Y; Zhong, Y; Zhang, X; Yu, Y; Yu, Z (2016). The "adaptive responses" of low concentrations of HBCD in L02 cells and the underlying molecular mechanisms Chemosphere, 145 68-76
Data Type:	Comet assay for HBCD
HERO ID:	3350502

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Cytotoxicity Data	Medium	× 1	2	Cytotoxicity was defined. Methods were briefly described and partially cited to manufacturer's instructions. Sampling was adequate; cell viability was measured indirectly (spectrophotometrically).
	Metric 25: Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡		High		1.5	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 36: **In vitro evaluation results of Huang et al 2016 for DNA damage**

Study Citation:	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
Data Type:	DNA damage for HBCD diastereomers
HERO ID:	3545979

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified by name (alpha-, beta-, and gamma-HBCD).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substances (a manufacturer) was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using a concurrent negative control group (DMSO-only) in which all conditions equal except exposure to test substance.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described (and appear appropriate), and partially cited to another publication (i.e., Tice et al. 2000 for the comet assay).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The study indicates that working solutions were freshly prepared. Storage conditions were not reported, but are not expected to substantially impact the study results owing to the short duration of the experiments (i.e., 24 and 48 hours for cell viability, 24 hours for comet assay).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appeared to be appropriate for the study type (e.g., 24 hours for comet assay).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups (3 doses plus controls for cell viability and comet assays) was reported. Although a rationale for dose selection was not provided, the highest dose used in the comet assay did not induce excessive cytotoxicity (as recommended).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.

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Study Citation:	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
Data Type:	DNA damage for HBCD diastereomers
HERO ID:	3545979

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 4: Test Model					
Metric 14:	Test Model	High	× 2	2	The cell types used were appropriate for the intended outcomes. The source of the cells was reported, and information such as media and number of passages was provided.
Metric 15:	Number per Group	High	× 1	1	An adequate number of replicates was reported (i.e., at least triplicate experiments/replicates for cell viability and comet assays).
Domain 5: Outcome Assessment					
Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodologies were described in detail.
Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes assessments were conducted consistently across study groups.
Metric 18:	Sampling Adequacy	High	× 2	2	The study indicates that three hundred cells per sample were evaluated (comet assay).
Metric 19:	Blinding of Assessors	High	× 1	1	It was indicated that cells used for analysis of DNA migration (comet assay) were randomly captured.
Domain 6: Confounding / Variable Control					
Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were identified.
Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis					
Metric 22:	Data Analysis	High	× 1	1	Statistical analyses were performed and appropriate for the study type.
Metric 23:	Data Interpretation	High	× 2	2	The study used statistical analyses to evaluate differences among study groups (and identify positive responses). It can also be inferred from the text that the time- and dose-relatedness of the response was considered (e.g., for cell viability and comet assays).
Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity was defined and methods were adequately described.
Metric 25:	Reporting of Data	High	× 2	2	Outcome data were reported by exposure group (means +/- standard deviations).
Overall Quality Determination‡		High		1.2	
Extracted		Yes			

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Study Citation:	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
Data Type:	DNA damage for HBCD diastereomers
HERO ID:	3545979

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

7 Developmental and Reproductive

Table 37: **Animal toxicity evaluation results of van der Ven 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, and hepatic outcomes**

Study Citation:	van der Ven, LTM; van de Kuil, T; Leonards, PEG; Slob, W; Lilienthal, H; Litens, S; Herlin, M; Håkansson, H; Cantón, RF; van den Berg, M; Visser, TJ; van Loveren, H; Vos, JG; Piersma, AH (2009). Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats Toxicology Letters, 185(1), 51-62
Data Type:	1-generation reproduction study, oral dietary
HERO ID:	589273

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified definitively as HBCD a mixture of three diastereoisomers, H alpha-, beta-, and gamm- HBCD and their respective proportion in the used batch was 10.3–8.7–81.0%.
	Metric 2:	Test Substance Source	Medium	× 1	2	The test substance manufacturer and source was reported; however, the batch/lot number was not specified.
	Metric 3:	Test Substance Purity	High	× 1	1	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.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	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.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not rated/applicable for this study type
	Metric 6:	Randomized Allocation	Low	× 1	3	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.
Domain 3: Exposure Characterization						

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Study Citation: van der Ven, LTM; van de Kuil, T; Leonards, PEG; Slob, W; Lilienthal, H; Litens, S; Herlin, M; Håkansson, H; Cantón, RF; van den Berg, M; Visser, TJ; van Loveren, H; Vos, JG; Piersma, AH (2009). Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats Toxicology Letters, 185(1), 51-62

Data Type: 1-generation reproduction study, oral dietary

HERO ID: 589273

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	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.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and administration was consistent between across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The targeted dietary exposure was reported to be 0–0.1–0.3–1–3–10–30–100 mg/kg bodyweight/day.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency (ad libitum) and duration of exposure were reported and appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and spacing was reported and was justified based on a preceding subacute repeated oral dose study.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route (oral, dietary) was reported and suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	The test animal species, strain, sex, and age was 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.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were reported and included temperature, humidity, and light-dark cycle. Husbandry conditions were adequate and the same for all animals.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was reported and appropriate for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology reported and sensitive to the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment methodology were reported and consistent across study groups for the outcomes of interest.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding the sampling for the outcomes of interest were reported and adequate for assessment.

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Study Citation:	van der Ven, LTM; van de Kuil, T; Leonards, PEG; Slob, W; Lilienthal, H; Litens, S; Herlin, M; Håkansson, H; Cantón, RF; van den Berg, M; Visser, TJ; van Loveren, H; Vos, JG; Piersma, AH (2009). Endocrine effects of hexabromocyclododecane (HBCD) in a one-generation reproduction study in Wistar rats Toxicology Letters, 185(1), 51-62
Data Type:	1-generation reproduction study, oral dietary
HERO ID:	589273

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not rated when outcomes are not subjective or for initial histopathology review.
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the negative control group was adequate. As shown in Supplemental tables 1-16 (ID2919529)
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	There were no reported differences among the study groups that could influence the outcome assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	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.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was shown for all datasets as evaluated for Supplemental tables 1-16 (ID2919529). BMD methodology was clearly described and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group and sex - as evaluated for Supplemental tables 1-16 (ID2919529).
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 38: **Animal toxicity evaluation results of Hachisuka et al 2010 for oral developmental immunotoxicity study on hematological and immune outcomes**

Study Citation:	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
Data Type:	Oral developmental immunotoxicity
HERO ID:	1403765

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Test substance identified by name.
	Metric 2:	Test Substance Source	Low	× 1	3	Source not identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Composition and purity not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control animals are included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not required.
	Metric 6:	Randomized Allocation	Low	× 1	3	Allocation methods were not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Limited details on preparation (mixed into the food) and no information on storage and stability were reported.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Animals were allowed to feed freely on the diet, but no details on the amount of diet provided was reported.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure duration was reported.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups and spacing were reported, but not justified.
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route and method were appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The species, strain, and sex were reported. The source and starting body weight of dams were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Details were not reported.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group was appropriate.
Domain 5: Outcome Assessment						

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Study Citation:	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
Data Type:	Oral developmental immunotoxicity
HERO ID:	1403765

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology was reported for some outcomes- hematology, thymus and spleen weight and pathology, and immunity. Other outcomes assessment mthodology, including body weight and weight gain, were not reported.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Sampling for some outcomes was not reported or illegible.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control responses were appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food/water intake of same were not reported and appear not to have been measured.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	There were not reported differences among the groups in health outcomes unrelated to exposures.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Statistical methods were not described but were conducted, and data were provided to conduct an independent analysis.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data were reported by groups, however it appears that not all outcomes were reported by sex.
Overall Quality Determination‡			Medium		2.0	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 39: **Animal toxicity evaluation results of Miller-Rhodes et al 2014 for developmental study and gestation day 1-parturition study on growth (early life) and development, and neurological/behavior outcomes**

Study Citation:	Miller-Rhodes, P; Popescu, M; Goeke, C; Tirabassi, T; Johnson, L; Markowski, VP (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
Data Type:	Developmental study; GD 1-parturition
HERO ID:	2528337

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Name and product number provided
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source
	Metric 3:	Test Substance Purity	High	× 1	1	Purity >95%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Use of vehicle control
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not necessary
	Metric 6:	Randomized Allocation	High	× 1	1	Randomized block design
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Prepared fresh daily, properly mixed.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure consistent across groups
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	concentrations were reported
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Daily gavage
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Three dose groups and a control
	Metric 12:	Exposure Route and Method	High	× 1	1	Gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Standard animal model used (Long Evans rats)
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry was reported and acceptable
	Metric 15:	Number per Group	High	× 1	1	10-11 pregnant dams/treatment group. (litters culled to 8 pups using randomized selection procedure)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methods were appropriate
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups

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Study Citation:	Miller-Rhodes, P; Popescu, M; Goeke, C; Tirabassi, T; Johnson, L; Markowski, VP (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
Data Type:	Developmental study; GD 1-parturition
HERO ID:	2528337

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	Low	× 1	3	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.
	Metric 19:	Blinding of Assessors	High	× 1	1	Stated that observers were blind to the exposure group
	Metric 20:	Negative Control Response	Medium	× 1	2	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.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	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
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	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.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	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.

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Study Citation:	Miller-Rhodes, P; Popescu, M; Goeke, C; Tirabassi, T; Johnson, L; Markowski, VP (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
Data Type:	Developmental study; GD 1-parturition
HERO ID:	2528337

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	Low	× 2	6	No individual offspring animal data were reported, therefore the data cannot be independently reviewed. Additionally, most data is 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.
Overall Quality Determination‡		~~High~~ ⟶ Medium§		~~1.4~~	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "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 was 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"

Table 40: **Animal toxicity evaluation results of Szabo et al 2016 for single dose gavage (PND 10) study in mice on metabolomics outcomes**

Study Citation:	Szabo, DT; Pathmasiri, W; Sumner, S; Birnbaum, LS (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
Data Type:	Single gavage in mice on PND 10; metabolomics evaluation only
HERO ID:	3546063

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chemical identity is clear; CAS #. provided Test substance is a commercial mixture of three stereoisomers. Percentages of each isomer are provided.
	Metric 2:	Test Substance Source	High	× 1	1	Sourced from Sigma-Aldrich
	Metric 3:	Test Substance Purity	Medium	× 1	2	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.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate negative (vehicle) control was used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control was not required.
	Metric 6:	Randomized Allocation	Low	× 1	3	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.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	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 were not necessary.

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Study Citation:	Szabo, DT; Pathmasiri, W; Sumner, S; Birnbaum, LS (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
Data Type:	Single gavage in mice on PND 10; metabolomics evaluation only
HERO ID:	3546063

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Dosing was equivalent across treatment groups (all animals given 10 mL/kg gavage of appropriate treatment)
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were clearly stated
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single exposure via gavage
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	An explanation of chosen doses was provided
	Metric 12:	Exposure Route and Method	High	× 1	1	Gavage was appropriate for pups that were still lactating.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Study clearly explains reasoning for choosing mice at this stage of development.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Animal husbandry conditions were appropriate.
	Metric 15:	Number per Group	Low	× 1	3	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.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	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 metabolimics, and no power analysis was done to determine an appropriate sample size. It is not clear whether technical replicates were included in the methodology.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment appeared to be consistent across groups

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Study Citation:	Szabo, DT; Pathmasiri, W; Sumner, S; Birnbaum, LS (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
Data Type:	Single gavage in mice on PND 10; metabolomics evaluation only
HERO ID:	3546063

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 18:	Sampling Adequacy	Low	× 1	3	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.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not indicated, but not necessarily applicable to NMR analysis
	Metric 20:	Negative Control Response	High	× 1	1	The responses of the controls are presumed to be appropriate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	The study authors did 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 were taken into account in the study design by treating across litters.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	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.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data presentation was adequate and appropriate for omics reporting. - Some data was presented in supplementary tables that were not available to view
Overall Quality Determination[‡]			~~High~~ ⟶ Medium[§]		~~1.5~~	

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Study Citation:	Szabo, DT; Pathmasiri, W; Sumner, S; Birnbaum, LS (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
Data Type:	Single gavage in mice on PND 10; metabolomics evaluation only
HERO ID:	3546063

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "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."

Table 41: **Animal toxicity evaluation results of Ema et al 2008 study on reproductive, growth (early life) and development, hepatic, neurological/behavior, and thyroid outcomes**

Study Citation:	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
Data Type:	
HERO ID:	787657

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The CASRN, purity, mixture components, and ratios were explicitly specified.
	Metric 2:	Test Substance Source	High	× 1	1	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.
	Metric 3:	Test Substance Purity	High	× 1	1	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).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	An appropriate concurrent control group was used (all of the conditions the same except exposure).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not indicated by study type.
	Metric 6:	Randomized Allocation	High	× 1	1	The study indicates that rats were randomly assigned into study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	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).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Analysis of the diet indicated that the test substance was administered at the desired feed concentrations throughout the study. Animals were fed ad libitum.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	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 premating, mating, gestation, lactation, and for the whole period of administration) were reported without ambiguity..
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	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).

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Study Citation: 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

Data Type:

HERO ID: 787657

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	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.
	Metric 12:	Exposure Route and Method	High	× 1	1	The test substance was administered in the diet (oral route is recommended by guideline).
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	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).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	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.
	Metric 15:	Number per Group	High	× 1	1	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.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcomes (mirrored guideline recommendations for a two-generation reproductive toxicity assay).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcomes were measured consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Reporting details were provided; litter data were recorded. Sampling was adequate for the outcomes of interest.
	Metric 19:	Blinding of Assessors	High	× 1	1	Although the study does not indicate that investigators were blinded to treatment group, the study cited various quality control methods that were followed.
	Metric 20:	Negative Control Response	High	× 1	1	The response of the negative controls was reported and were adequate (e.g. there were no histological findings in the thyroid of control rats).

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Study Citation: 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

Data Type:

HERO ID: 787657

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences in initial body weights or intake that could influence the outcome assessment.
	Metric 22: Health Outcomes Unrelated to Exposure	High	× 1	1	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.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Statistical methods were clearly described.
	Metric 24: Reporting of Data	High	× 2	2	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).
Overall Quality Determination‡		High		1.0	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 42: **Animal toxicity evaluation results of Eriksson et al 2006 for oral neurodevelopmental study (single dose PND 10) study on neurological/behavior, and growth (early life) and development outcomes**

Study Citation:	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
Data Type:	Oral neurodevelopmental study (single dose PND10)
HERO ID:	787660

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Characterized as a mixture containing three diastereo-isomers alpha-, beta-, and gamma-HBCD.
	Metric 2:	Test Substance Source	Low	× 1	3	Prepared from a commercial mixture, but the manufacturer and lot/batch number were not given. Analytical verification is not described.
	Metric 3:	Test Substance Purity	High	× 1	1	>98%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative vehicle controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not needed for neurodevelopmental studies.
	Metric 6:	Randomized Allocation	High	× 1	1	Randomly selected from 3-4 different litters from each treatment group.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation was well described and appropriate. Single dose study, therefore prolonged storage is not a concern.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	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).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Gavage doses were reported as both mg/kg and umol/kg.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Administered as single dose during a critical period (on PND 10) in neonatal development of the mouse brain.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 doses plus control. A justification was not provided for the doses selected, but the results suggest they were appropriate..
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain and age of neonatal mice was specified.

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Study Citation: 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
Data Type: Oral neurodevelopmental study (single dose PND10)
HERO ID: 787660

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	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.
	Metric 15:	Number per Group	High	× 1	1	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)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Standard tests of spontaneous behavior and learning and memory.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	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.
	Metric 18:	Sampling Adequacy	Low	× 1	3	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. If litters were assessed, then metric 15 (number of animals per group) would be insufficient.
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported; however, outcomes were objective.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group(s) were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no significant deviations in body weight gain in HBCDD-treated mice compared with the vehicle-treated mice.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						

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Study Citation:	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
Data Type:	Oral neurodevelopmental study (single dose PND10)
HERO ID:	787660

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 23: Statistical Methods	Low	× 1	3	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.
	Metric 24: Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group and sex.
Overall Quality Determination‡		~~High~~ ⟶ Medium§		~~1.4~~	
Extracted		No			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

§ Evaluator's explanation for rating change: "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)"

Table 43: **Animal toxicity evaluation results of Lilienthal et al 2009 for 1-generation reproductive study, dietary exposure study on neurological/behavior outcomes**

Study Citation:	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
Data Type:	1-generation reproductive study, dietary exposure
HERO ID:	787693

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Isomer composition of HBCD was reported.
	Metric 2:	Test Substance Source	Medium	× 1	2	Supplier was Bromine Science and Environmental Forum. No information on lot or batch and no analytical verification was described.
	Metric 3:	Test Substance Purity	High	× 1	1	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.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Untreated and vehicle controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not needed for neurobehavioral studies.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of test diets was described; however, the frequency of preparation and store was not indicated.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and exposures were administered consistently across study groups in a scientifically sound manner.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose in mg/kg/day were calculated by study authors.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Continuous paternal and maternal exposure during premating, mating, gestation, lactation and after weaning in offspring was reported.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose/concentration spacing were justified by study authors and considered adequate to address the purpose of the study.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.

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Study Citation:	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
Data Type:	1-generation reproductive study, dietary exposure
HERO ID:	787693

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain, sex and starting age were provided (commercial source).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Husbandry conditions were reported and appropriate.
	Metric 15:	Number per Group	High	× 1	1	6/sex/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed or reported the intended outcome(s) of interest and was sensitive for the outcomes(s) of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcome(s) of interest were reported.
	Metric 19:	Blinding of Assessors	High	× 1	1	The authors report that "personnel conducting the measurements were unaware of the exposure conditions" suggesting the assessors were blinded.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group(s) were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food/water intake were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistics and BMD modeling was reported.
	Metric 24:	Reporting of Data	High	× 2	2	Test data and BMD results were reported.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	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
Data Type:	1-generation reproductive study, dietary exposure
HERO ID:	787693

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 44: **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 Citation:	Saeguas, Y., Fujimoto, H., Woo, G.H., Inoue, K., Takahashi, M., Mitsumori, K., Hirose, M., Nishikawa, A. (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
Data Type:	1-Generation Developmental Toxicity (Dietary Exposure)
HERO ID:	787721

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer and lot no. were reported..
	Metric 3:	Test Substance Purity	High	× 1	1	>95%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control not needed developmental studies.
	Metric 6:	Randomized Allocation	High	× 1	1	Randomized allocation.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	Test substance preparation and storage were not described.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported as mg/kg-day (mean +/- SD) for 3 time periods (GD 10-20, PND 1-9 and PND 10-20)
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Daily exposure during critical developmental periods.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Range-finding study was used to set doses.. 3 treatment groups plus controls.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and were suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Test animals were obtained from a commercial source. Species, strain, and preganancy status were reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported and appropriate.

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Study Citation:	Saeguas, Y., Fujimoto, H., Woo, G.H., Inoue, K., Takahashi, M., Mitsumori, K., Hirose, M., Nishikawa, A. (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
Data Type:	1-Generation Developmental Toxicity (Dietary Exposure)
HERO ID:	787721

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	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).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Thorough outcome examinations pubertal and adult necropsies).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment protocol were reported and outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	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).
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported, but outcomes were objective.
	Metric 20:	Negative Control Response	High	× 1	1	No histopathology lesion in controls.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences among groups in food consumption and body weight.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition and/or health outcomes unrelated to exposure were not reported for each study group
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were clearly described and appropriate for dataset(s).
	Metric 24:	Reporting of Data	Medium	× 2	4	HBCD caused a dose-dependent decrease in Cingulate deep cortex CNPase (+) cell count, which was significantly lower at the highest dose exposed.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	Saeguas, Y., Fujimoto, H., Woo, G.H., Inoue, K., Takahashi, M., Mitsumori, K., Hirose, M., Nishikawa, A. (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
Data Type:	1-Generation Developmental Toxicity (Dietary Exposure)
HERO ID:	787721

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

8 Mechanistic

Table 45: **In vitro evaluation results of Anisuzzaman and Whalen 2016 for secretion of IL-1beta**

Study Citation:	Anisuzzaman, S; Whalen, MM (2016). Tetrabromobisphenol A and hexabromocyclododecane alter secretion of IL-1ß from human immune cells Journal of Immunotoxicology, 13(3), 403-416
Data Type:	Secretion of IL-1beta for HBCD
HERO ID:	3350463

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance (a manufacturer) was identified; not information on batch/lot number was provided.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative (vehicle-only) controls were included for each cell type/study condition.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not required. However, treatment-related positive responses were observed (i.e., demonstrating the test is capable of detecting a positive response).
	Metric 6:	Assay Procedures	High	× 1	1	The study authors described the methods and procedures (e.g., test conditions, cell density, culture media, temperatures) used for the test in detail and they were applicable for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	The study indicates that stock solutions were made by dissolving the test substance in DMSO; concentrations used in the assays were prepared by diluting the stock solution, Information about stability and storage were not reported (for studies as long as 6 days in duration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The study used varying duration of exposures (24 hours, 48 hours, 6 days).

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Study Citation:	Anisuzzaman, S; Whalen, MM (2016). Tetrabromobisphenol A and hexabromocyclododecane alter secretion of IL-1ß from human immune cells Journal of Immunotoxicology, 13(3), 403-416
Data Type:	Secretion of IL-1beta for HBCD
HERO ID:	3350463

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups was reported (as many as 7 groups plus controls), and the rationale for selected doses was reported (i.e., ranges tested were based those that had caused effects on NK lytic function, cell-surface protein expression, and MAPK activity).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test models (natural killer cells, monocyte-depleted peripheral blood mononuclear cells [PMBC], and PBMC) was reported along with limited descriptive information. The sources of cells was reported. The cell types were appropriate for the outcome of interest because they secrete pro-inflammatory cytokines.
	Metric 15:	Number per Group	High	× 1	1	The study indicates that experiments were performed in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported in adequate detail.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	The study authors acknowledged differences in the response of cells to HBCD for various donors; data were shown for individual donors.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No reported outcome differences among study groups unrelated to exposure were reported (not expected to impact the study results).
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were clearly described and presented for datasets of interest (i.e., ANOVA and Student's t-tests).
	Metric 23:	Data Interpretation	Medium	× 2	4	Statistical significance served as the primary criteria for a positive response.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cell viability was defined and methods were described.

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Study Citation:	Anisuzzaman, S; Whalen, MM (2016). Tetrabromobisphenol A and hexabromocyclododecane alter secretion of IL-1ß from human immune cells Journal of Immunotoxicology, 13(3), 403-416
Data Type:	Secretion of IL-1beta for HBCD
HERO ID:	3350463

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	All data were reported by exposure group.
Overall Quality Determination‡		High		1.3	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 46: **In vitro evaluation results of Wang et al 2016 for metabolic pathways for mechanism of toxicity**

Study Citation:	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
Data Type:	Metabolic pathways for mechanism of toxicity for HBCD
HERO ID:	3350479

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	The test substance was clearly identified by name.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance was reported (without information regarding batch/lot number).
	Metric 3:	Test Substance Purity	High	× 1	1	Reported purity and grade (reagent-grade; >95% pure) were such that effects likely due to test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative control (vehicle-only) groups were included; the only difference among groups was exposure to the test substance.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type. However, treatment-related positive responses were observed (i.e., demonstrating the test is capable of detecting a positive response).
	Metric 6:	Assay Procedures	High	× 1	1	The study authors described the methods and procedures (e.g., test conditions, culture media and volumes, temperatures) used for the test in detail and they were applicable for the study type. Some methodological information was provided in the Supporting Information. Although a non-traditional method was used for metabolomic analysis (psuedotargeted approach rather than Q-TOF MS untargeted method), the authors showed that their method produced results that were repeatable.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The study indicates that HBCD stock solutions were prepared in DMSO and incorporated into the cell culture medium. Information on stability and storage were not reported (but are not expected to impact the study results owing to the short duration of the study [24 hours for the metabolomic portion of the study]).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity.

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Study Citation:	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
Data Type:	Metabolic pathways for mechanism of toxicity for HBCD
HERO ID:	3350479

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Durations were reported and adequate for the study type. The duration of the metabolomic study was based on results for the cell viability assay.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of groups (3 groups plus controls) was reported and based on cell viability testing (lowest dose with observable inhibition effect, middle dose without observable effect, and highest dose with observable stimulation effect). The lowest dose was also comparable with the maximum serum concentration of HBCD in occupationally exposed individuals.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (HepG2 cells) and descriptive information were reported, the test model was obtained from a commercial source (China Infrastructure of Cell Line Resources. The study indicates that this test system was used as a model because the cells are stable, have an unlimited lifespan, and retains liver-specific functions. In addition, its molecular expression and biological phenotypes have been extensively characterized.
	Metric 15:	Number per Group	High	× 1	1	The number of replicates (n = 6 for metabolomic analyses) was appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodologies were described in adequate detail. Some of this information is reported in the Supplemental Information.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables in test design and procedures were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables in outcomes unrelated to exposure were reported (none are expected to impact the study results).
Domain 7: Data Presentation and Analysis						

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Study Citation:	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
Data Type:	Metabolic pathways for mechanism of toxicity for HBCD
HERO ID:	3350479

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods (e.g., ANOVA using statistics software) and data manipulation (with respect to metabolomic analyses) were reported and appropriate for the study type.
	Metric 23:	Data Interpretation	High	× 2	2	The study indicated the criteria for positive responses (e.g., based on statistical significance or dose-response).
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoints were defined and methods were reported in adequate detail.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for outcomes by exposure group.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 47: **In vitro evaluation results of Kim et al 2016 for cancer progression (cell growth, apoptosis, migration, gene expression)**

Study Citation:	Kim, SH; Nam, KH; Hwang, KA; Choi, KC (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
Data Type:	Cancer progression (cell growth, apoptosis, migration, gene expression) for HBCD
HERO ID:	3350494

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified by name.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance (a manufacturer) was identified (no batch/lot numbers provided).
	Metric 3:	Test Substance Purity	High	× 1	1	Purity was reported (>99% or analytical standard); therefore, effects observed are likely due to test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent vehicle-only control groups were used; conditions appeared to be equal other than addition of the test substance.
	Metric 5:	Positive Controls	High	× 2	2	Dihydrotestosterone was used as a positive control (as a potent androgen that binds strongly to the androgen receptor [AR]). The study aimed to evaluate whether HBCD enhances prostate cancer progression via AR in vitro.
	Metric 6:	Assay Procedures	High	× 1	1	Methods and procedures were described in adequate detail (e.g., cell density, culture media and volumes, temperature).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Test substance was dissolved in solvent (DMSO) but no other details (e.g., storage) were provided. The absence of details is considered a deficiency owing to the duration of experiments conducted in this study (i.e., up to 5 days in duration for the migration assay).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration is listed for all experiments (and are considered appropriate for the study type(s).

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Study Citation:	Kim, SH; Nam, KH; Hwang, KA; Choi, KC (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
Data Type:	Cancer progression (cell growth, apoptosis, migration, gene expression) for HBCD
HERO ID:	3350494

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of exposure groups was reported (as many as 4 dose groups plus controls for viability, as few as 1 dose group for migration and gene expression assays). It was indicated that doses were in the range of human/environmental exposure levels. The doses used did not generate a dose-related effect (viability assay); and dose-relatedness could not be evaluated for experiments using only one dose.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was appropriate for the outcomes of interest (i.e., cancer progression endpoints via androgen receptor endpoints in prostatic cancer cells). The number of passages were indicated; however, the source of the cells was not specified.
	Metric 15:	Number per Group	High	× 1	1	The study indicated that each experiment was repeated three times.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodologies were reported in adequate detail. However, it is noted that the approach used to evaluate gene expression was semi-quantitative (based on band intensity).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analyses used in the analyses were reported. In the figure legends, it is indicated when Dunnett's comparison test was used (unclear if Student's t-test was applied to the dataset(s).

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Study Citation:	Kim, SH; Nam, KH; Hwang, KA; Choi, KC (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
Data Type:	Cancer progression (cell growth, apoptosis, migration, gene expression) for HBCD
HERO ID:	3350494

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	Medium	× 2	4	Statistical analyses were used as the basis for positive results. Dose-relatedness could only be assessed for the cell viability assay (the only experiment that utilized multiple dose levels); effects on cell viability were not dose-related.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Methods to determine cell viability were described in adequate detail.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all outcomes by exposure group.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 48: **In vitro evaluation results of Koike et al 2016 for immune response in respiratory cells**

Study Citation:	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
Data Type:	Immune response in respiratory cells for HBCD
HERO ID:	3350501

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance identified by name, structure, and molecular weight.
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance (a manufacturer) was reported (without information on a batch/lot number).
	Metric 3:	Test Substance Purity	High	× 1	1	The reported purity of the test substance (>95%) was such that observed effects are likely due to the test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative (vehicle-only) controls were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type. Treatment with then substances used in the study induced positive responses (indicative of the efficacy of the test system). For the nuclear receptor-ligand binding assay, 3,3',5-triiodo-L-thyronine (T3) was used as a positive control for the thyroid receptor and beta-estradiol was used as a positive control for the estrogen receptor.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were partially reported (immunoassay for EGFR phosphorylation, transciption factor assay) and partially cited to other publications and/or protocols (measurement of the expression of pro-inflammatory proteins, nuclear receptor-ligand binding assay).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation details were reported (i.e., test substance was diluted in DMSO), but storage and stability conditions were not reported (not expected to impact the study results owing to the short duration of the experiments [up to 24 hours]).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.

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Study Citation:	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
Data Type:	Immune response in respiratory cells for HBCD
HERO ID:	3350501

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported. The range of concentrations was reported in the methods; individual concentrations were shown or could be estimated from graphs and/or figure legends.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The duration of exposures was clearly reported for most assays; however, the duration of the ligand-binding assay was not explicitly specified.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of groups (1 to 3 dose groups plus controls depending on assay type) was reported. A rationale for the selected doses was not provided, and it was not clear how exposure concentrations were selected when only one dose group was used. The dose used in the assays evaluating the expression of pro-inflammatory proteins and activation of transcription factors also statistically significantly induced toxicity (albeit < viability was >80% of controls).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported with minimal descriptive information. Human respiratory cells were used because inhalation is expected to be a relevant route of exposure. The bronchial epithelial BEAS-2B cells were obtained from a cell culture collection. These cells are not routinely used for the outcomes of interest.
	Metric 15:	Number per Group	High	× 1	1	The study indicates that results were from triplicate cultures and experiments were repeated 2 or 3 times.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodologies were described for some assays, but some of the information for others (e.g., EGFR phosphorylation, transcription factor assay, nuclear-receptor-ligand binding) was cited to manufacturer's protocols.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently among study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						

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Study Citation:	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
Data Type:	Immune response in respiratory cells for HBCD
HERO ID:	3350501

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variable in assay design were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables in outcomes were reported (and none are expected to impact the study results).
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	The statistical methods used were reported and appeared to be appropriate for the study types.
	Metric 23:	Data Interpretation	Medium	× 2	4	It appeared that statistical significance was the primary determinant of a positive response. Some of the assays only used one dose (therefore, the dose-relatedness of responses could not be evaluated).
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cell viability endpoints were defined, described, and appropriate for the study type.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group. Negative findings were reported qualitatively (e.g., nuclear receptor-ligand binding assay).
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 49: **In vitro evaluation results of Wu et al 2016 for cardiac toxicity**

Study Citation:	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
Data Type:	Cardiac toxicity for HBCD
HERO ID:	3350515

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified clearly by name. A structure was also provided (in the graphical abstract).
	Metric 2:	Test Substance Source	Medium	× 1	2	The source of the test substance (a manufacturer) was reported (no batch/lot number was provided).
	Metric 3:	Test Substance Purity	High	× 1	1	The reported purity (95%) was such that effects likely due to the test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative (vehicle-only) control groups were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study type. Treatment-related positive responses were observed for the test substance (i.e., demonstrating the test is capable of detecting a positive response).
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures (measurement of calcium transients, gene expression analyses) were described and appropriate. Minor details were cited to manufacturer's instructions.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was described (i.e., dissolved in DMSO), but storage and/or stability was not adequately reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The duration of exposure was reported (24 hours for Ca2+ handling, and 24 to 36 hours for gene expression studies) and appeared to be appropriate for the study type(s).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups was reported (three groups plus controls for Ca2+ handling, one for gene expression analyses). The rationale for dose selection was not provided; however, it was inferred that doses were based on earlier studies.

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Study Citation:	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
Data Type:	Cardiac toxicity for HBCD
HERO ID:	3350515

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (rat cardiomyocyte cell line H9C2) and source were reported and were appropriate for the study type (i.e., evaluating cardiac toxicity).
	Metric 15:	Number per Group	High	× 1	1	The number of replicates (n =6) was reported in the figure legends (see Figures 2 and 5).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodologies were reported and appropriate. Minor details were cited to manufacturer's instructions.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables in test design were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables in outcomes unrelated to exposure were reported (and not expected to impact the study results).
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were reported and appropriate for the study types.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	The dose-relatedness and statistical significance of effects on Ca2+ handling were considered. For gene expression, statistical analyses and fold-changes were evaluated.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric not applicable to the study type.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡			High		1.2	
Extracted			Yes			

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Study Citation:	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
Data Type:	Cardiac toxicity for HBCD
HERO ID:	3350515

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.
†† This metric met the criteria for high confidence as expected for this type of study

Table 50: **In vitro evaluation results of Almughamsi and Whalen 2016 for altered inflammatory cytokine in human cells**

Study Citation:	Almughamsi, H; Whalen, MM (2016). Hexabromocyclododecane and tetrabromobisphenol A alter secretion of interferon gamma (IFN-<U+03B3>) from human immune cells Archives of Toxicology, 90(7), 1695-1707
Data Type:	Altered inflammatory cytokine in human cells
HERO ID:	3350524

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	Source was identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity/grade and/or composition were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent controls were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not required.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were reported.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	No standards were required for the assays.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Limited preparation details were provided and not storage or stability data were reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Durations were reported.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups and spacing were reported but not justified.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not required for the assay.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test models and sources were identified and appropriate.
	Metric 15:	Number per Group	High	× 1	1	The number of cells exposure were reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was reported.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each study replicate or group.

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Study Citation:	Almughamsi, H; Whalen, MM (2016). Hexabromocyclododecane and tetrabromobisphenol A alter secretion of interferon gamma (IFN-<U+03B3>) from human immune cells Archives of Toxicology, 90(7), 1695-1707
Data Type:	Altered inflammatory cytokine in human cells
HERO ID:	3350524

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables in outcomes unrelated to exposures were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were reported and appropriate.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Metric not required.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cell viability methods were defined and described.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported.
Overall Quality Determination‡			High		1.3	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 51: **In vitro evaluation results of Canbaz et al 2016 for immune effects**

Study Citation:	Canbaz, D; Lebre, MC; Logiantara, A; van Ree, R; van Rijt, LS (2016). Indoor pollutant hexabromocyclododecane enhances house dust mite-induced activation of human monocyte-derived dendritic cells Journal of Immunotoxicology, 13(6), 1-7
Data Type:	Immune effects
HERO ID:	3355511

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name.
	Metric 2:	Test Substance Source	High	× 1	1	Source identified.
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance described as technical mixture, but purity/grade and/or composition were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not required.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were reported..
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Standards not required for assays.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Limited preparation details were reported, but stability and storage were not.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were administered consistently.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Durations were reported and appropriate.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of groups and spacing were reported nut not justified.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Activation not required.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model and donor information were provided.
	Metric 15:	Number per Group	Medium	× 1	2	The number of cells per group in the initial exposure assay was not reported, but was reported for the cytokine assay.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was .
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	High	× 2	2	Sampling was adequate.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not required.
Domain 6: Confounding / Variable Control						

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Study Citation:	Canbaz, D; Lebre, MC; Logiantara, A; van Ree, R; van Rijt, LS (2016). Indoor pollutant hexabromocyclododecane enhances house dust mite-induced activation of human monocyte-derived dendritic cells Journal of Immunotoxicology, 13(6), 1-7
Data Type:	Immune effects
HERO ID:	3355511

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each study replicate or group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Two donors did not yield sufficient cells to perform all experiments.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were reported and appropriate.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Data interpretation criteria not required.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Methods were not reported but the data were provided.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Epidemiological Studies

Table Listing

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Table 1: **Roze et al. 2009: Evaluation of Neurological/Behavior Outcomes**

Study Citation:	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
Data Type:	GIC cohort HBCD_coordination outcome-Neurological/Behavior
HERO ID:	758049

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Study Participation						
	Metric 1:	Participant selection	Medium	× 0.4	0.8	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.
	Metric 2:	Attrition	High	× 0.4	0.4	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).
	Metric 3:	Comparison Group	Medium	× 0.2	0.4	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.
Domain 2: Exposure Characterization						
	Metric 4:	Measurement of Exposure	High	× 0.4	0.4	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.

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Study Citation: 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

Data Type: GIC cohort HBCD_coordination outcome-Neurological/Behavior

HERO ID: 758049

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 5:	Exposure levels	Medium	× 0.2	0.4	Range (0.3-7.5 ng/g lipid) and distribution (continuous) of exposure is sufficient to establish an exposure response estimate.
	Metric 6:	Temporality	Medium	× 0.4	0.8	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.
Domain 3: Outcome Assessment						
	Metric 7:	Outcome measurement or characterization	High	× 0.667	0.67	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.
	Metric 8:	Reporting Bias	Low	× 0.333	1.0	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.
Domain 4: Potential Counfounding/Variable Control						
	Metric 9:	Covariate Adjustment	Medium	× 0.5	1	Results were adjusted for some covariates (such as SES, HOME, and sex) without providing a description of methods.
	Metric 10:	Covariate Characterization	Medium	× 0.25	0.5	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.

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Study Citation:	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
Data Type:	GIC cohort HBCD_coordination outcome-Neurological/Behavior
HERO ID:	758049

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Co-exposure Confounding	Medium	× 0.25	0.5	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.
Domain 5: Analysis						
	Metric 12:	Study Design and Methods	Medium	× 0.4	0.8	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.
	Metric 13:	Statistical power	Medium	× 0.2	0.4	The number of participants (i.e., 62) seem adequate to detect an effect in the exposed population.
	Metric 14:	Reproducibility of analyses	Low	× 0.2	0.6	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.
	Metric 15:	Statistical models	Medium	× 0.2	0.4	As described, it appears that the method is appropriate and that assumptions were met (or data were transformed).
Domain 6: Other Considerations for Biomarker Selection and Measurement						
	Metric 16:	Use of Biomarker of Exposure	High	× 0.2	0.2	Maternal serum levels of HBCD is a biomarker in a specified matrix that has accurate and precise relationship with external exposure.
	Metric 17:	Effect biomarker	Not Rated	NA	NA	No biomarker of effect was measured.
	Metric 18:	Method Sensitivity	Medium	× 0.2	0.4	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 adequatrly reported. LOD/LOQ (value or %) are reported.
	Metric 19:	Biomarker stability	Low	× 0.2	0.6	No information was provided on storage history or stability.
	Metric 20:	Sample contamination	Medium	× 0.2	0.4	There is incomplete documentation of the steps taken to provide necessary assurance that the study data are reliable.
	Metric 21:	Method requirements	Medium	× 0.2	0.4	Instrumentation provides unambiguous identification and quantification of the biomarker at the require sensitivity (GC-MS).

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Study Citation:	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
Data Type:	GIC cohort HBCD_coordination outcome-Neurological/Behavior
HERO ID:	758049

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 22: Matrix adjustment	Not Rated	NA	NA	I don't think any adjustment is needed.
Overall Quality Determination‡		Medium		1.8	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 2: **Eggesbø et al., 2011: Evaluation of Thyroid Outcomes**

Study Citation:	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
Data Type:	Q3 vs Q1 HBCD and neonatal TSH levels-Thyroid
HERO ID:	787656

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Study Participation					
	Metric 1: Participant selection	High	× 0.4	0.4	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.
	Metric 2: Attrition	Medium	× 0.4	0.8	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.
	Metric 3: Comparison Group	High	× 0.2	0.2	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.
Domain 2: Exposure Characterization					
	Metric 4: Measurement of Exposure	High	× 0.4	0.4	High rating: exposure was assessed using the same well-established methods that directly measure HBCD in breast milk, a frequently used biomarker of exposure.

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Study Citation: 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
Data Type: Q3 vs Q1 HBCD and neonatal TSH levels-Thyroid
HERO ID: 787656

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 5:	Exposure levels	Medium	× 0.2	0.4	Medium rating: range and distribution of exposure was sufficient to develop an exposure-response estimate; 3 or more levels of exposure were reported.
	Metric 6:	Temporality	High	× 0.4	0.4	High rating: temporality is established and the interval between the exposure and the outcome has an appropriate consideration of relevant exposure windows.
Domain 3: Outcome Assessment						
	Metric 7:	Outcome measurement or characterization	High	× 0.667	0.67	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).
	Metric 8:	Reporting Bias	High	× 0.333	0.33	High rating: all of the study's measured outcomes are reported, effect estimates reported with confidence interval; number of exposed reported for each analysis.
Domain 4: Potential Counfounding/Variable Control						
	Metric 9:	Covariate Adjustment	High	× 0.5	0.5	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.
	Metric 10:	Covariate Characterization	Medium	× 0.25	0.5	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.
	Metric 11:	Co-exposure Confounding	Medium	× 0.25	0.5	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,
Domain 5: Analysis						
	Metric 12:	Study Design and Methods	Medium	× 0.4	0.8	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.

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Study Citation:	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
Data Type:	Q3 vs Q1 HBCD and neonatal TSH levels-Thyroid
HERO ID:	787656

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Statistical power	Medium	× 0.2	0.4	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.
	Metric 14:	Reproducibility of analyses	Medium	× 0.2	0.4	Medium rating: description of the analyses is sufficient to understand what has been done and to be reproducible with access to the data.
	Metric 15:	Statistical models	Medium	× 0.2	0.4	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.
Domain 6: Other Considerations for Biomarker Selection and Measurement						
	Metric 16:	Use of Biomarker of Exposure	High	× 0.143	0.14	High rating: Evidence exists for a relationship between HBCD in breast milk and external exposure.
	Metric 17:	Effect biomarker	High	× 0.143	0.14	High rating: Effect biomarker measured is an indicator of a key event in an AOP.
	Metric 18:	Method Sensitivity	Medium	× 0.143	0.29	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.
	Metric 19:	Biomarker stability	High	NA	NA	High rating: samples with a known storage history (Supplement-03 document)
	Metric 20:	Sample contamination	Low	× 0.143	0.43	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.
	Metric 21:	Method requirements	High	× 0.143	0.14	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.
	Metric 22:	Matrix adjustment	Medium	× 0.143	0.29	Medium rating: study only provides results using one method (lipid-adjusted).
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	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
Data Type:	Q3 vs Q1 HBCD and neonatal TSH levels-Thyroid
HERO ID:	787656

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 3: **Meijer et al. 2012: Evaluation of Reproductive for sex hormone outcomes Outcomes**

Study Citation:	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
Data Type:	GIC cohort HBCD_sex hormones-Reproductive
HERO ID:	1401499

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Study Participation					
Metric 1:	Participant selection	Medium	× 0.4	0.8	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.
Metric 2:	Attrition	High	× 0.4	0.4	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.
Metric 3:	Comparison Group	Medium	× 0.2	0.4	HBCD was evaluated on a continuous basis and there is no indication that there was anything different about the exposure in this cohort.
Domain 2: Exposure Characterization					
Metric 4:	Measurement of Exposure	High	× 0.4	0.4	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.
Metric 5:	Exposure levels	Medium	× 0.2	0.4	Range (not detected to 7.4 ng/g lipid) and distribution (continuous) of exposure is sufficient to establish an exposure response estimate.

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Study Citation:	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
Data Type:	GIC cohort HBCD_sex hormones-Reproductive
HERO ID:	1401499

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 6:	Temporality	Medium	× 0.4	0.8	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).
Domain 3: Outcome Assessment						
	Metric 7:	Outcome measurement or characterization	Medium	× 0.667	1.33	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.
	Metric 8:	Reporting Bias	Low	× 0.333	1.0	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.
Domain 4: Potential Counfounding/Variable Control						
	Metric 9:	Covariate Adjustment	Low	× 0.667	2	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.
	Metric 10:	Covariate Characterization	Not Rated	NA	NA	Covariates were not assessed.
	Metric 11:	Co-exposure Confounding	Medium	× 0.333	0.67	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.
Domain 5: Analysis						
	Metric 12:	Study Design and Methods	Medium	× 0.4	0.8	The study design chosen was appropriate for the research question. The study used an appropriate statistical method to address the research question.
	Metric 13:	Statistical power	Medium	× 0.2	0.4	The number of participants (i.e., 55) seem adequate to detect an effect in the exposed population.

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Study Citation:	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
Data Type:	GIC cohort HBCD_sex hormones-Reproductive
HERO ID:	1401499

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Reproducibility of analyses	Medium	× 0.2	0.4	The description of the analysis is sufficient to understand precisely what was done and to be conceptually reproducible with access to the analytic data.
	Metric 15:	Statistical models	Medium	× 0.2	0.4	There is a clear description of the analyses.
Domain 6: Other Considerations for Biomarker Selection and Measurement						
	Metric 16:	Use of Biomarker of Exposure	High	× 0.167	0.17	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.
	Metric 17:	Effect biomarker	Medium	× 0.167	0.33	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).
	Metric 18:	Method Sensitivity	Medium	× 0.167	0.33	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.
	Metric 19:	Biomarker stability	Medium	× 0.167	0.33	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.
	Metric 20:	Sample contamination	Medium	× 0.167	0.33	There is incomplete documentation of the steps taken to provide the necessary assurance that the study data are reliable.
	Metric 21:	Method requirements	High	× 0.167	0.17	Instrumentation that provides unambiguous identification and quantitation of the biomarker at the required sensitivity (GC–MS) was used.
	Metric 22:	Matrix adjustment	Not Rated	NA	NA	I don't think this is applicable to either matrix measured.
Overall Quality Determination‡			Medium		2.0	
Extracted			Yes			

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Study Citation:	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
Data Type:	GIC cohort HBCD_sex hormones-Reproductive
HERO ID:	1401499

Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor
† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.
‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 4: **Meijer et al. 2012: Evaluation of Reproductive for male sexual development outcomes Outcomes**

Study Citation:	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
Data Type:	GIC cohort HBCD_male sexual development-Reproductive
HERO ID:	1401499

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Study Participation					
Metric 1:	Participant selection	Medium	× 0.4	0.8	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.
Metric 2:	Attrition	High	× 0.4	0.4	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.
Metric 3:	Comparison Group	Medium	× 0.2	0.4	HBCD was evaluated on a continuous basis and there is no indication that there was anything different about the exposure in this cohort.
Domain 2: Exposure Characterization					
Metric 4:	Measurement of Exposure	High	× 0.4	0.4	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.

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Study Citation:	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
Data Type:	GIC cohort HBCD_male sexual development-Reproductive
HERO ID:	1401499

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 5:	Exposure levels	Medium	× 0.2	0.4	Range (not detected to 7.4 ng/g lipid) and distribution (continuous) of exposure is sufficient to establish an exposure response estimate.
	Metric 6:	Temporality	Medium	× 0.4	0.8	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).
Domain 3: Outcome Assessment						
	Metric 7:	Outcome measurement or characterization	High	× 0.667	0.67	Testes volume was measured by ultrasound. Measurements were preformed 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.
	Metric 8:	Reporting Bias	Low	× 0.333	1.0	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.
Domain 4: Potential Counfounding/Variable Control						
	Metric 9:	Covariate Adjustment	Low	× 0.667	2	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.
	Metric 10:	Covariate Characterization	Not Rated	NA	NA	Covariates were not assessed.

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Study Citation:	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
Data Type:	GIC cohort HBCD_male sexual development-Reproductive
HERO ID:	1401499

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Co-exposure Confounding	Medium	× 0.333	0.67	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.
Domain 5: Analysis						
	Metric 12:	Study Design and Methods	Medium	× 0.4	0.8	The study design chosen was appropriate for the research question. The study used an appropriate statistical method to address the research question.
	Metric 13:	Statistical power	Medium	× 0.2	0.4	The number of participants (i.e., 55) seem adequate to detect an effect in the exposed population.
	Metric 14:	Reproducibility of analyses	Medium	× 0.2	0.4	The description of the analysis is sufficient to understand precisely what was done and to be conceptually reproducible with access to the analytic data.
	Metric 15:	Statistical models	Medium	× 0.2	0.4	There is a clear description of the analyses.
Domain 6: Other Considerations for Biomarker Selection and Measurement						
	Metric 16:	Use of Biomarker of Exposure	High	× 0.167	0.17	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.
	Metric 17:	Effect biomarker	Medium	× 0.167	0.33	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).
	Metric 18:	Method Sensitivity	Medium	× 0.167	0.33	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.

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Study Citation:	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
Data Type:	GIC cohort HBCD_male sexual development-Reproductive
HERO ID:	1401499

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 19:	Biomarker stability	Medium	× 0.167	0.33	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.
	Metric 20:	Sample contamination	Medium	× 0.167	0.33	There is incomplete documentation of the steps taken to provide the necessary assurance that the study data are reliable.
	Metric 21:	Method requirements	High	× 0.167	0.17	Instrumentation that provides unambiguous identification and quantitation of the biomarker at the required sensitivity (GC–MS) was used.
	Metric 22:	Matrix adjustment	Not Rated	NA	NA	I don't think this is applicable to either matrix measured.
Overall Quality Determination‡			Medium		1.9	
Extracted			Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 5: **Johnson et al. 2013: Evaluation of Reproductive Outcomes**

Study Citation:	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
Data Type:	Cross-sectional, HBCD Exposed males house dust, endpoint sex hormone binding globulin-Reproductive
HERO ID:	1676758

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Study Participation					
	Metric 1: Participant selection	High	× 0.25	0.25	No explanation for participation rate of 65% provided; only male subjects. Information on participation selection, inclusion and exclusion criteria are provided in cited publications.
	Metric 2: Attrition	Low	× 0.4	1.2	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.
	Metric 3: Comparison Group	Unacceptable	× 0.2	0.04	There is no information on a comparison group. However correlation analysis performed looking for trend on a continuum of exposure.
Domain 2: Exposure Characterization					
	Metric 4: Measurement of Exposure	Medium	× 0.4	0.8	Dust samples were collected from used vacuum badge from home. It is unclear if this is an established method to to determine levels of exposure. HBCD detected in 97% of samples.
	Metric 5: Exposure levels	Low	× 0.2	0.6	The range of exposure is limited but based on the analysis it does allow limited exploration in the exposure-response relationship.
	Metric 6: Temporality	Medium	× 0.4	0.8	Dust samples and serum hormone levels are sampled in the same year for participants. The temporality of exposure and outcome is uncertain.
Domain 3: Outcome Assessment					
	Metric 7: Outcome measurement or characterization	High	× 0.667	0.67	QA/QC methods described in another paper. The outcome was assessed using established methods.
	Metric 8: Reporting Bias	Medium	× 0.333	0.67	Author's discuss results in text for significant results only
Domain 4: Potential Counfounding/Variable Control					
	Metric 9: Covariate Adjustment	High	× 0.5	0.5	Although models were adjusted for age and BMI for some flame retardants, there is no mention of covariate consideration for HBCD.
	Metric 10: Covariate Characterization	High	× 0.25	0.25	There is no information to suggest that the questionnaire used was validated; however there is no evidence that the method had poor validity.

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Study Citation:	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
Data Type:	Cross-sectional, HBCD Exposed males house dust, endpoint sex hormone binding globulin-Reproductive
HERO ID:	1676758

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Co-exposure Confounding	Medium	× 0.25	0.5	Cannot rule out possibility of that findings are due to unmeasured co-exposures (e.g. other chemicals in household dust).
Domain 5: Analysis						
	Metric 12:	Study Design and Methods	Unacceptable	× 0.4	0.16	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.
	Metric 13:	Statistical power	Unacceptable	× 0.2	0.04	The sample size is relatively small and the authors indicate that the study is exploratory in nature.
	Metric 14:	Reproducibility of analyses	Medium	× 0.2	0.4	The analysis is sufficiently described.
	Metric 15:	Statistical models	Medium	× 0.2	0.4	The authors provide an explanation for when data is combined with previous study data and limitations of the analysis in detail.
Domain 6: Other Considerations for Biomarker Selection and Measurement						
	Metric 16:	Use of Biomarker of Exposure	Not Rated	NA	NA	No biomarker of exposure measured.
	Metric 17:	Effect biomarker	Unacceptable	× 0.25	0.06	Biomarker not specific to a health outcome.
	Metric 18:	Method Sensitivity	Not Rated	NA	NA	Limit of detection not discussed in study, but no evidence of insufficient data.
	Metric 19:	Biomarker stability	High	NA	NA	samples with known storage history and documented stability data
	Metric 20:	Sample contamination	Medium	× 0.25	0.5	No information to indicate sample contamination.
	Metric 21:	Method requirements	High	× 0.25	0.25	Method provides the identification and quantification of the biomarker.
	Metric 22:	Matrix adjustment	Not Rated	NA	NA	No matrix adjustment.
Overall Quality Determination‡			Unacceptable⋆⋆		2.1	
Extracted			No			

⋆⋆ Consistent with our *Application of Systematic Review in TSCARisk 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 or more of the metrics were rated as unacceptable. As such, the study is considered unacceptable and the score is presented solely to increase transparency.

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 6: **Kicinski et al. 2012: Evaluation of Neurological/Behavior Outcomes**

Study Citation:	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 86
Data Type:	HBCD finger taps (change in number of taps with non-preferred hand)-Neurological/Behavior
HERO ID:	1927571

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Study Participation						
	Metric 1:	Participant selection	High	× 0.4	0.4	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 adeolescents 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-responsers were noted to not be different from the responders except that there was a higher proportion of girls responding.
	Metric 2:	Attrition	Medium	× 0.4	0.8	107 of the 606 subjects included were excluded because of missing covariates (n=84), missing blood measurements (n=3), or did not complete neurobehavioral tests (n=4). However, results have much fewer numbers for some results without full explanation.
	Metric 3:	Comparison Group	Medium	× 0.2	0.4	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.
Domain 2: Exposure Characterization						
	Metric 4:	Measurement of Exposure	Low	× 0.4	1.2	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.
	Metric 5:	Exposure levels	Low	× 0.2	0.6	For HBCD the effects of the concentrations above the LOQ compared to the concentrations below the LOQ were estimated (binary exposure).

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Study Citation:	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 86
Data Type:	HBCD finger taps (change in number of taps with non-preferred hand)-Neurological/Behavior
HERO ID:	1927571

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 6:	Temporality	Low	× 0.4	1.2	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.
Domain 3: Outcome Assessment						
	Metric 7:	Outcome measurement or characterization	High	× 0.667	0.67	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.
	Metric 8:	Reporting Bias	High	× 0.333	0.33	Sufficient information is provided. All outcomes were reported with effect, 95% confidence intervals, and sample size.
Domain 4: Potential Counfounding/Variable Control						
	Metric 9:	Covariate Adjustment	High	× 0.5	0.5	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 consumotion, 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.
	Metric 10:	Covariate Characterization	Medium	× 0.25	0.5	Information was obtained via questionnaires some information to be filled out by the adolescent and some for the parents.
	Metric 11:	Co-exposure Confounding	Medium	× 0.25	0.5	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.
Domain 5: Analysis						

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Study Citation:	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 86
Data Type:	HBCD finger taps (change in number of taps with non-preferred hand)-Neurological/Behavior
HERO ID:	1927571

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Study Design and Methods	Medium	× 0.4	0.8	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.
	Metric 13:	Statistical power	Medium	× 0.2	0.4	Sufficient statistical power with 515 included subjects and outcome results available for 340 to 511 for any specific outcome.
	Metric 14:	Reproducibility of analyses	Low	× 0.2	0.6	Description is not 100% clear on methods to be reproducible.
	Metric 15:	Statistical models	Medium	× 0.2	0.4	The use of a linear regression or a negative binomial model were acceptable for the data with assumptions met or data transformed.
Domain 6: Other Considerations for Biomarker Selection and Measurement						
	Metric 16:	Use of Biomarker of Exposure	Medium	× 0.2	0.4	No information is provided to indicate serum HBCD is the appropriate, but the parent compound was measured.
	Metric 17:	Effect biomarker	Not Rated	NA	NA	No biomarker of effect was measured.
	Metric 18:	Method Sensitivity	Low	× 0.2	0.6	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.
	Metric 19:	Biomarker stability	Medium	× 0.2	0.4	No information was provided on storage history or stability of the HBCD in the sample.
	Metric 20:	Sample contamination	Medium	× 0.2	0.4	There is incomplete documentation of the steps taken to provide the necessary assurance that the study data are reliable.
	Metric 21:	Method requirements	Medium	× 0.2	0.4	Solid phase extraction followed by gas chromatography mass spectometry in electron capture negative ion mode was used. Specifics of the extraction were not provided, but are assumed 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.
	Metric 22:	Matrix adjustment	Not Rated	NA	NA	Don't think matrix adjustment would be appropriate for this biomarker of exposure.
Overall Quality Determination‡			Medium		1.9	

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Study Citation:	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 86
Data Type:	HBCD finger taps (change in number of taps with non-preferred hand)-Neurological/Behavior
HERO ID:	1927571

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High =≥ 1 to < 1.7; Medium =≥ 1.7 to < 2.3; Low =≥ 2.3 to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 7: **Kicinski et al. 2012: Evaluation of Thyroid Outcomes**

Study Citation:	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 86
Data Type:	HBCD T3 change-Thyroid
HERO ID:	1927571

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Study Participation						
	Metric 1:	Participant selection	High	× 0.4	0.4	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 adeolescents 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-responsers were noted to not be different from the responders except that there was a higher proportion of girls responding.
	Metric 2:	Attrition	Medium	× 0.4	0.8	107 of the 606 subjects included were excluded because of missing covariates (n=84), missing blood measurements (n=3), or did not complete neurobehavioral tests (n=4). However, results have much fewer numbers for some results without full explanation.
	Metric 3:	Comparison Group	Medium	× 0.2	0.4	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.
Domain 2: Exposure Characterization						
	Metric 4:	Measurement of Exposure	Low	× 0.4	1.2	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.
	Metric 5:	Exposure levels	Low	× 0.2	0.6	For HBCD the effects of the concentrations above the LOQ compared to the concentrations below the LOQ were estimated (binary exposure).

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Study Citation:	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 86
Data Type:	HBCD T3 change-Thyroid
HERO ID:	1927571

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 6:	Temporality	Low	× 0.4	1.2	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.
Domain 3: Outcome Assessment						
	Metric 7:	Outcome measurement or characterization	Medium	× 0.667	1.33	Thyroid hormones were measured by competitive immune assays. No other information was provided. These are assumed to be standard assays.
	Metric 8:	Reporting Bias	Medium	× 0.333	0.67	Information is provided, but not enough for complete extraction (sample size was not specified).
Domain 4: Potential Counfounding/Variable Control						
	Metric 9:	Covariate Adjustment	High	× 0.5	0.5	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 consumotion, 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.
	Metric 10:	Covariate Characterization	Medium	× 0.25	0.5	Information was obtained via questionnaires some information to be filled out by the adolescent and some for the parents.
	Metric 11:	Co-exposure Confounding	Medium	× 0.25	0.5	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.
Domain 5: Analysis						
	Metric 12:	Study Design and Methods	Medium	× 0.4	0.8	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.
	Metric 13:	Statistical power	Medium	× 0.2	0.4	Sufficient statistical power with 515 included subjects.

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Study Citation: 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 86

Data Type: HBCD T3 change-Thyroid

HERO ID: 1927571

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 14:	Reproducibility of analyses	Low	× 0.2	0.6	Description is not 100% clear on methods to be reproducible.
	Metric 15:	Statistical models	Medium	× 0.2	0.4	The use of a linear regression or a negative binomial model were acceptable for the data with assumptions met or data transformed.
Domain 6: Other Considerations for Biomarker Selection and Measurement						
	Metric 16:	Use of Biomarker of Exposure	Medium	× 0.167	0.33	No information is provided to indicate serum HBCD is the appropriate, but the parent compound was measured.
	Metric 17:	Effect biomarker	Low	× 0.167	0.5	Biomarkers of effect shown to have a relationship to health outcomes, but the method is not well validated and mechanism of action is not understood.
	Metric 18:	Method Sensitivity	Low	× 0.167	0.5	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.
	Metric 19:	Biomarker stability	Medium	× 0.167	0.33	No information was provided on storage history or stability of the HBCD or thyroid hormones in the sample.
	Metric 20:	Sample contamination	Medium	× 0.167	0.33	There is incomplete documentation of the steps taken to provide the necessary assurance that the study data are reliable.
	Metric 21:	Method requirements	Medium	× 0.167	0.33	Solid phase extraction followed by gas chromatography mass spectometry in electron capture negative ion mode was used. Specifics of the extraction were not provided, but are assumed 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.
	Metric 22:	Matrix adjustment	Not Rated	NA	NA	Don't think matrix adjustment would be appropriate for this biomarker of exposure or thyroid hormones.
Overall Quality Determination‡			Medium		2.1	
Extracted			Yes			

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Data Type:	HBCD T3 change-Thyroid
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Domain	Metric	Rating†	MWF⋆	Score	Comments††

⋆ MWF = Metric Weighting Factor

† High = 1; Medium = 2; Low = 3; Unacceptable = 4; N/A has no value.

‡ The overall rating is calculated as necessary. EPA may not always provide a comment for a metric that has been categorized as High.

$$\text{Overall rating} = \begin{cases} 4 & \text{if any metric is Unacceptable} \\ \left\lfloor \sum_i (\text{Metric Score}_i \times \text{MWF}_i) \,/\, \sum_j \text{MWF}_j \right\rceil_{0.1} & \text{(round to the nearest tenth) otherwise} \end{cases},$$

where High $=\geq 1$ to < 1.7; Medium $=\geq 1.7$ to < 2.3; Low $=\geq 2.3$ to ≤ 3.0. If the reviewer determines that the overall rating needs adjustment, the original rating is crossed out and an arrow points to the new rating.

†† This metric met the criteria for high confidence as expected for this type of study

Table 8: **Kim and Oh 2014: Evaluation of Thyroid Outcomes**

Study Citation:	Kim, UJ; Oh, JE (2014). Tetrabromobisphenol A and hexabromocyclododecane flame retardants in infant-mother paired serum samples, and their relationships with thyroid hormones and environmental factors Environmental Pollution, 184 193-200
Data Type:	HBCDs Mother & Infants THs (T3)-Thyroid
HERO ID:	2324769

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Study Participation					
	Metric 1: Participant selection	Low	× 0.4	1.2	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 elegibility 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).
	Metric 2: Attrition	High	× 0.4	0.4	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.
	Metric 3: Comparison Group	Medium	× 0.2	0.4	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.
Domain 2: Exposure Characterization					
	Metric 4: Measurement of Exposure	High	× 0.4	0.4	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.

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Data Type: HBCDs Mother & Infants THs (T3)-Thyroid
HERO ID: 2324769

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 5:	Exposure levels	Medium	× 0.2	0.4	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.
	Metric 6:	Temporality	Low	× 0.4	1.2	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 do not give an accurate indication of prenatal exposure and it's relationship to congenital hypothyroidism. Thus, the temporality of exposure and outcome is uncertain.
Domain 3: Outcome Assessment						
	Metric 7:	Outcome measurement or characterization	High	× 0.667	0.67	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.
	Metric 8:	Reporting Bias	Medium	× 0.333	0.67	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.
Domain 4: Potential Counfounding/Variable Control						
	Metric 9:	Covariate Adjustment	Low	× 0.667	2	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.
	Metric 10:	Covariate Characterization	Not Rated	NA	NA	Covariates were not assessed.
	Metric 11:	Co-exposure Confounding	Medium	× 0.333	0.67	Other brominated flame retardants were measured and reported in this study. There is no indication of differential exposure between cases and controls.
Domain 5: Analysis						

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Data Type: HBCDs Mother & Infants THs (T3)-Thyroid
HERO ID: 2324769

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Study Design and Methods	Medium	× 0.5	1	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.
	Metric 13:	Statistical power	Medium	× 0.25	0.5	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.
	Metric 14:	Reproducibility of analyses	Medium	× 0.25	0.5	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.
	Metric 15:	Statistical models	Not Rated	NA	NA	No statistical model used.
Domain 6: Other Considerations for Biomarker Selection and Measurement						
	Metric 16:	Use of Biomarker of Exposure	High	× 0.143	0.14	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.
	Metric 17:	Effect biomarker	High	× 0.143	0.14	TSH, T4, and other thyroid hormone levels are appropriate measures of thyroid conditions.
	Metric 18:	Method Sensitivity	Medium	× 0.143	0.29	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.
	Metric 19:	Biomarker stability	High	NA	NA	No apparent issues; storage history is documented.
	Metric 20:	Sample contamination	High	× 0.143	0.14	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).
	Metric 21:	Method requirements	High	× 0.143	0.14	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).

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Data Type:	HBCDs Mother & Infants THs (T3)-Thyroid
HERO ID:	2324769

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 22: Matrix adjustment	Medium	× 0.143	0.29	HBCDs in serum are presented only as matrix adjusted (ng/g lipid).
Overall Quality Determination‡		Medium		1.9	
Extracted		Yes			

⋆ MWF = Metric Weighting Factor

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