United States
Environmental Protection Agency

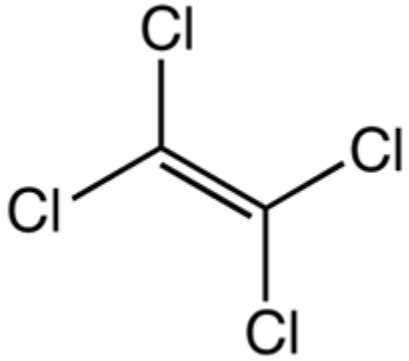
Office of Chemical Safety and
Pollution Prevention

Final Risk Evaluation for Perchloroethylene

Systematic Review Supplemental File:

Data Quality Evaluation of Human Health Hazard Studies – Animal and *in Vitro* Studies

CASRN: 127-18-4



December 2020

Table Listing

Acute (<24 hr)

Short-term (1-30 days)

Dermal Absorption

Subchronic (30-90 days)

Chronic (>90 days)

Genetic toxicity studies

Developmental and Reproductive

Mechanistic

1 Acute (<24 hr)

Table 1: **Animal toxicity evaluation results of Dow et al 1950 for an acute and repeat inhalation exposures study on mortality, body weight, respiratory, cardiovascular, hepatic, renal, hematological and immune, reproductive, neurological/behavior, endocrine, gastrointestinal, musculoskeletal, ocular and sensory outcomes**

Study Citation:	Dow Chemical Company (1950). Vapor toxicity of tetrachloroethylene for laboratory animals and human subjects
Data Type:	Acute and Repeat Inhalation exposures
HERO ID:	4214242

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Tetrachoroethylene identified by name and structure.
	Metric 2:	Test Substance Source	Medium	× 1	2	"* samples of commercial product" - manufacturer not identified. Confirmed identity in lab.
	Metric 3:	Test Substance Purity	High	× 1	1	99.9%C
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	No controls reported for acute studies. In repeat-exposure study, authors indicated untreated and air-exposed controls were used "for each experiment". It is not clear if they were all concurrent because exposure duration varied drastically in different exposure groups within the same species.
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	Low	× 1	3	Animals were "carefully selected on the basis of general appearance, body weight, and growth during a preliminary period of observation".
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Vaporization method reported with limited details. Storage not reported.
	Metric 8:	Consistency of Exposure Administration	Unacceptable	× 1	4	Exposure durations varied widely between exposure groups within the same species (unclear if each duration had a concurrent control group). Only guinea pigs had two exposure groups (and presumably a control group) with the same duration (exposed 14 days over an 18 day period) for meaningful dose-response analysis (but data reporting inadequate for analysis). Different chambers were used for different concentrations in repeat-exposure studies.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Only target levels were reported. Air concentrations were monitored, and reportedly within 10% of target

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Study Citation: Dow Chemical Company (1950). Vapor toxicity of tetrachloroethylene for laboratory animals and human subjects
Data Type: Acute and Repeat Inhalation exposures
HERO ID: 4214242

Domain		Metric	Rating†	MWF*	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	Exposure at different concentrations in acute studies ranged from minutes to 14 hours. Exposure at different concentrations in repeat exposure studies (7 hr/d, 5 d/wk) ranged from 18-236d for various species.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Acute exposure:: 4 exposure levels, no control. (lack of control addressed in prior Metric 4, not here) Repeated exposure: All exposure groups except Monkeys had at lease 2 exposure groups plus control. With the exception of 2 (of 4) guinea pig groups, exposure groups were not directly comparable due to different exposure durations.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	Acute: glass, 160L, air rate of 15-30 L/min (which equates 6-12 air changes per hour).. Animals in groups of 5-12. Repeat: Metal chamber about 450L for 100 ppm, metal chamber of 1700 L for 200 and 400 ppm, glass chamber of 160L for 1600 and 2500 ppm. Air flow rate not reported.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Rat: Internal albino colony originally obtained from Wistar Institute of Anatomy and Biology in 1938 Gn Pig: Heterogeneous stock purchased from "commercial breeder" Rabbit: Albino, internal heterogeneous colony (no further details) Monkey: Rhesus - "newly imported", no further details. No ages reported for any species. Initial BW data only available graphically for a couple exposure groups.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Diet for each species reported. No other husbandry conditions reported.
	Metric 15:	Number per Group	Medium	× 1	2	Acute: 5-30 per dose per duration Repeat: Rat: 5-22/sex per group Rabbit: 2/sex per group Guinea Pig: 5-15/sex per group Monkey - 2 M/group Number varied widely between exposure groups.
Domain 5: Outcome Assessment						

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Study Citation: Dow Chemical Company (1950). Vapor toxicity of tetrachloroethylene for laboratory animals and human subjects
Data Type: Acute and Repeat Inhalation exposures
HERO ID: 4214242

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Acute: Mortality, clinical signs, hepatic injury Repeat: Mortalty, clinical signs, BW, select organ weight and histology, hematology in some animals
	Metric 17:	Consistency of Outcome Assessment	Unacceptable	× 1	4	Acute: Timing different across exposure groups. Unclear for repeat exposure - all animals were evaluated for mortality, CS, BW, OW, and "organic injury" - assuming gross necropsy; periodic hematology was performed on "several groups of animals", not further defined; clinical chemistry was evaluated in "many cases"; in "many instances" organs were examined histologically. Depending on which groups were evaluated, timing was different due to different exposure durations between exposure levels.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Unclear how many animals were evaluated for several of the metrics (see Metric 17)
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	
	Metric 20:	Negative Control Response	Low	× 1	3	Data reporting limited. Where exposure group data were reported quantitatively, control data were included. Remaining data reported qualitatively (change or no change from control).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Acute: Anaesthetic effects with unconsciousness and failure of respiration in acute study at all exposures except the lowest (2000 ppm) Repeat: CNS depression also reported at highest concentration (2500 ppm) in rat, mice, GP (no mention of respiratory depression)
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Acute- no statistics, data for mortality adequate for independent analsysi Repeat: t-test was reported used "wherever possible"" Reported only for guinea pig group exposed to 0 or 200 ppm for "as many as 158 Seven-hour Exposures in 220 days"
	Metric 24:	Reporting of Data	Low	× 2	6	Only limited data sets were reported quantitatively, the majority were reported qualitatively only (even with exposure-related effects)
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		2.6	

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Study Citation:	Dow Chemical Company (1950). Vapor toxicity of tetrachloroethylene for laboratory animals and human subjects
Data Type:	Acute and Repeat Inhalation exposures
HERO ID:	4214242

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 2: **Animal toxicity evaluation results of Dow et al 1983 for an acute dermal lethality study in rabbits on mortality and irritation outcomes**

Study Citation:	Dow Chemical Company (1983). Initial submission: Perchloroethylene solvent formulation: acute toxicological properties & industrial handling hazards, with cover letter dated 102591 (sanitized)
Data Type:	acute dermal lethality study in rabbits
HERO ID:	4214440

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Low	× 2	6	Test substance identity was reported by unambiguous name, and reference was made to an appendix containing the composition, but the table was blanked out in the appendix in the pdf.
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance source was reported, but without certification or analytical verification of identity.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported; reference was made to an appendix containing the composition, but the table was blanked out in the appendix in the pdf.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Negative controls not common in lethality studies
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for this study type.
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	There was only one group
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	No information on preparation or storage of test material was provided.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Volume and skin surface area of application were not reported.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Exposure reported as mg/kg. Initial body weights were not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure was for 24 hours which is adequate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only one dose (200 mg/kg) was tested, and it was well below the recommended dose for a limit test (2000 mg/kg). An attempt was made to test 2000 mg/kg but this dose resulted in significant animal pain.
	Metric 12:	Exposure Route and Method	High	× 1	1	Acute Percutaneous Absorption
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal source, species, strain, and sex were reported; age and initial body weight were not.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Housing conditions, acclimation, and photoperiod were reported, but temperature and humidity were not.

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Study Citation:	Dow Chemical Company (1983). Initial submission: Perchloroethylene solvent formulation: acute toxicological properties & industrial handling hazards, with cover letter dated 102591 (sanitized)
Data Type:	acute dermal lethality study in rabbits
HERO ID:	4214440

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	5 male rabbits were used; this number is consistent with guidelines.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Outcome assessment methodologies for mortality, body weight, and necropsy were reported. Irritation responses were described, but a scoring system was not applied.
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Only a single group was used.
	Metric 18:	Sampling Adequacy	High	× 1	1	Although the protocol called for only surviving animals to be necropsied, all exposed animals survived, so all were necropsied.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	As there was only one group blinding was not possible/necessary.
	Metric 20:	Negative Control Response	Not Rated	NA	NA	Negative controls not required for acute lethality test
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	No potentially confounding factors were identified, but initial health conditions were not reported.
	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	Not Rated	NA	NA	Statistical analysis is not possible on a single exposure group.
	Metric 24:	Reporting of Data	Medium	× 2	4	Data reporting was adequate for the type of study.
Overall Quality Determination‡			~~Unacceptable~~** ⟶ Low§		~~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 =≥ 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 reviewer upgraded this study's overall quality rating, changing its status from unacceptable to acceptable. They noted: The only metric that was unacceptable was test substance preparation and storage, which is of low concern for single dose dermal administration. Although a score was calculated, it is not presented here because the final rating was changed based on professional judgement."

Table 3: **Animal toxicity evaluation results of Dow et al 1983 for an acute dermal irritation study on irritation outcomes**

Study Citation:	Dow Chemical Company (1983). Initial submission: Perchloroethylene solvent formulation: acute toxicological properties & industrial handling hazards, with cover letter dated 102591 (sanitized)					
Data Type:	acute dermal irritation in rabbits					
HERO ID:	4214440					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Low	× 2	6	Test substance identity was reported by unambiguous name, and reference was made to an appendix containing the composition, but the table was blanked out in the appendix in the pdf.
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance source was reported, but without certification or analytical verification of identity.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported; reference was made to an appendix containing the composition, but the table was blanked out in the appendix in the pdf.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Negative control groups not required for dermal irritation test
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for this study type.
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	There was only one group
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	No information on preparation or storage of test material was provided.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Skin surface area tested was not reported.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Dermal patches were left in place for 24 hours which is adequate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Only one exposure level was tested, but it reflected the highest concentration (undiluted) possible.
	Metric 12:	Exposure Route and Method	High	× 1	1	
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal source, species, strain, and sex were reported; age and initial body weight were not.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Housing conditions, acclimation, and photoperiod were reported, but temperature and humidity were not.
	Metric 15:	Number per Group	High	× 1	1	6 rabbits were used; this is more than required for testing.
Domain 5: Outcome Assessment						

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Study Citation:	Dow Chemical Company (1983). Initial submission: Perchloroethylene solvent formulation: acute toxicological properties & industrial handling hazards, with cover letter dated 102591 (sanitized)
Data Type:	acute dermal irritation in rabbits
HERO ID:	4214440

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Unacceptable	× 2	8	Outcome assessment methodology was inadequately reported (lacking irritation scoring details)
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Only a single group was used.
	Metric 18:	Sampling Adequacy	High	× 1	1	All exposed animals were evaluated for all outcomes.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	As there was only one group blinding was not possible/necessary.
	Metric 20:	Negative Control Response	Not Rated	NA	NA	There was no negative control group.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	No potentially confounding factors were identified, but initial health conditions were not reported.
	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	Not Rated	NA	NA	Statistical analysis is not typical for this study type.
	Metric 24:	Reporting of Data	Unacceptable	× 2	8	Individual skin irritation scores were not reported.
Overall Quality Determination‡			Unacceptable⋆⋆		2.3	
Extracted			No			

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⋆ 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 4: **Animal toxicity evaluation results of Dow et al 1983 for an acute oral toxicity study in rats on mortality and acute toxicity/poisoning outcomes**

Study Citation:	Dow Chemical Company (1983). Initial submission: Perchloroethylene solvent formulation: acute toxicological properties & industrial handling hazards, with cover letter dated 102591 (sanitized)					
Data Type:	acute oral toxicity in rats					
HERO ID:	4214440					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Low	× 2	6	Test substance identity was reported by unambiguous name, and reference was made to an appendix containing the composition, but the table was blanked out in the appendix in the pdf.
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance source was reported, but without certification or analytical verification of identity.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported; reference was made to an appendix containing the composition, but the table was blanked out in the appendix in the pdf.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Negative controls not required for lethality studies.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	Study did not report how animals were allocated to groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	No information on preparation or storage of test material was provided.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Some details of exposure administration were not reported (e.g., gavage volume) but these are unlikely to affect the results.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported unambiguously as mg/kg bw
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Single exposure is typical for this study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	There were 5 nonzero exposure groups, and the maximum dose administered (5000 mg/kg) is commonly used in limit tests. Dose range and spacing were adequate to enable calculation of LD50 values with reasonable confidence limits.
	Metric 12:	Exposure Route and Method	High	× 1	1	Acute oral/gavage
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal source, species, strain, and sex were reported; age and initial body weight were not.

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Study Citation:	Dow Chemical Company (1983). Initial submission: Perchloroethylene solvent formulation: acute toxicological properties & industrial handling hazards, with cover letter dated 102591 (sanitized)
Data Type:	acute oral toxicity in rats
HERO ID:	4214440

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 14: Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Housing conditions, acclimation, and photoperiod were reported, but temperature and humidity were not.
	Metric 15: Number per Group	Medium	× 1	2	6 rats/sex/dose were used.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology was reported, but outcomes were limited to mortality, clinical signs, body weight, and gross necrospsy.
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	There were no reported inconsistencies in outcome assessment.
	Metric 18: Sampling Adequacy	High	× 1	1	All exposed animals were evaluated for all outcomes.
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	Most outcomes (apart from clinical signs) were not subjective.
	Metric 20: Negative Control Response	Not Rated	NA	NA	There was no negative control group.
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	Medium	× 2	4	No potentially confounding factors were identified, but food intake was not measured and could have affected body weights.
	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	Medium	× 1	2	Statistical analysis of lethality data was conducted, and data enabling independent analysis were reported.
	Metric 24: Reporting of Data	Medium	× 2	4	Mortality and clinical signs were reported in detail, including time of death/onset of symptoms, but body weights were not reported.
Overall Quality Determination[‡]		~~Unacceptable~~[⋆⋆] ⟶ Low[§]		~~2.0~~	
Extracted		Yes			

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Study Citation:	Dow Chemical Company (1983). Initial submission: Perchloroethylene solvent formulation: acute toxicological properties & industrial handling hazards, with cover letter dated 102591 (sanitized)
Data Type:	acute oral toxicity in rats
HERO ID:	4214440

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.

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

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: "The only metric that was unacceptable was test substance preparation and storage, which is of low concern for single dose gavage administration."

Table 5: **Animal toxicity evaluation results of Dow et al 1983 for an acute eye irritation study in rabbits on irritation outcomes**

Study Citation:	Dow Chemical Company (1983). Initial submission: Perchloroethylene solvent formulation: acute toxicological properties & industrial handling hazards, with cover letter dated 102591 (sanitized)
Data Type:	acute eye irritation in rabbits
HERO ID:	4214440

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Low	× 2	6	Test substance identity was reported by unambiguous name, and reference was made to an appendix containing the composition, but the table was blanked out in the appendix in the pdf.
	Metric 2:	Test Substance Source	Medium	× 1	2	Test substance source was reported, but without certification or analytical verification of identity.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity was not reported; reference was made to an appendix containing the composition, but the table was blanked out in the appendix in the pdf.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Negative control group not required for eye irritation tests; untreated eye serves as control
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for this study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	Animal allocation to study groups was not described.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	No information on preparation or storage of test material was provided.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Study does not clearly state that undiluted test material was used, but based on the language and approach to other experiments in the paper, it is likely that this is the case.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Exposure reported as volume of test material; concentration/purity of of Perc in test material was not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Dermal patches were left in place for 24 hours which is adequate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Single exposure level is acceptable for eye irritation testing.
	Metric 12:	Exposure Route and Method	High	× 1	1	Route and method are typical for this study type.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal source, species, strain, and sex were reported; age and initial body weight were not.

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Study Citation:	Dow Chemical Company (1983). Initial submission: Perchloroethylene solvent formulation: acute toxicological properties & industrial handling hazards, with cover letter dated 102591 (sanitized)
Data Type:	acute eye irritation in rabbits
HERO ID:	4214440

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Housing conditions, acclimation, and photoperiod were reported, but temperature and humidity were not.
	Metric 15:	Number per Group	High	× 1	1	9 rabbits were used; this is more than required for testing.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology was adequately reported; Draize scoring method was cited but scoring details not provided.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Two exposure groups were used (one with eyes rinsed after 30 sec and one with no rinsing)
	Metric 18:	Sampling Adequacy	High	× 1	1	All exposed animals were evaluated for all outcomes.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	As there was no control group blinding was not possible/necessary.
	Metric 20:	Negative Control Response	Not Rated	NA	NA	There was no negative control group.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No potentially confounding factors were identified. Eye condition was examined and determined to be healthy before testing.
	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	Not Rated	NA	NA	Statistical analysis is not typical for eye irritation tests.
	Metric 24:	Reporting of Data	High	× 2	2	Individual and group irritation scores for each time point were reported.
Overall Quality Determination‡			~~Unacceptable~~** ⟶ Low§		~~1.8~~	
Extracted			Yes			

** 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

§ Evaluator's explanation for rating change: "The only metric that was unacceptable was test storage and preparation which is of low concern in a single exposure eye irritation test."

Table 6: **Animal toxicity evaluation results of Beliles et al 1980 for acute inhalation studies on genotoxicity in vivo outcomes**

Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	acute inhalation studies
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name and synonym
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer and lot number given.
	Metric 3:	Test Substance Purity	Medium	× 1	2	91% pure, impurities were not characterized
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Filtered air controls; control animals exposed in a different room.
	Metric 5:	Positive Controls	High	× 1	1	Positive controls (reference mutagens) were used for all studies.
	Metric 6:	Randomized Allocation	High	× 1	1	randomly assigned to groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Method and equipment used to generate the test substance as a vapor were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and analytical concentrations were provided. Range of measure concentration did not deviate more than 10%.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Acute duration appropriate for dominant lethal and spermhead abnormality.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 exposure concentrations (100 and 500ppm)
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic chamber , whole body, assumed that Perc does not condense.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain and source were reported; starting age and bw not given.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	well reported
	Metric 15:	Number per Group	High	× 1	1	6-10/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Dominant lethal assay, spermhead abnormality, chromosomal aberration in rat bone marrow,

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Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	acute inhalation studies
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported, but most outcomes were not subjective.
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	None related to genotoxicity
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	None related to genotoxicity
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistics were well described and appropriate
	Metric 24:	Reporting of Data	High	× 2	2	All outcomes were reported.
Overall Quality Determination‡			High		1.2	
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

2 Short-term (1-30 days)

Table 7: **Animal toxicity evaluation results of NTP 1986 for 1-day inhalation studies in rats and mice on acute toxicity, neurological/behavioral, mortality, nutrition and metabolic/adult exposure body weight outcomes**

Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	1-d inhalation studies - rats and mice
HERO ID:	632655

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	high-purity tetrachloroethylene, Dowper stabilized
	Metric 2:	Test Substance Source	High	× 1	1	Dow Chemical, lot TA03116F-01. Purity and identity analyses conducted.
	Metric 3:	Test Substance Purity	High	× 1	1	Confirmed analytically - approximately 99.9%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Chamber controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed for study type.
	Metric 6:	Randomized Allocation	Medium	× 1	2	stratified by weight then assigned to groups according to a table of random numbers (weight is a non-random component)
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Tetrachloroethylene was found to be stable for 2 weeks at 60" C (Appendix H). Tetrachloroethylene was stored at 0" C Tetrachloroethylene was vaporized at 100"- 110" C, diluted with air, and. introduced into the chambers. Detailed descriptions in Table 2 and in Appendix I.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Concentrations in the exposure chambers were monitored 8-12 times per exposure period by a Hewlett-Packard 5840A Gas Chromatograph. No deviations from protocol noted.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Only target concentrations reported for non-chronic studies., but actual exposures expected to be close to target based on 2-yr analytical values.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	1-d, 4 hr
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	5 dose groups plus control
	Metric 12:	Exposure Route and Method	Low	× 1	3	Inhalation, dynamic whole-body chamber. Flow rate not reported
Domain 4: Test Organism						

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Study Citation: NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type: 1-d inhalation studies - rats and mice
HERO ID: 632655

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	High	× 2	2	F344/N rats and B6C3F1 mice, Frederick Cancer Research Center. 5-7 wks at study initiation. Initial body weights reported in Tables 6 and 17.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Some details of husbandry in Table 5; Room conditions not reported
	Metric 15:	Number per Group	High	× 1	1	5/sex/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Mortality, clinical signs, body weight, necropsy
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation in all study groups
	Metric 18:	Sampling Adequacy	High	× 1	1	5/sex/group
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Evaluated endpoints did not require blinding
	Metric 20:	Negative Control Response	High	× 1	1	Control responses reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no reported differences among the study groups in initial body weight. Food and water intake were not reported. Respiratory rate not reported, but severe clinical signs included anesthesia were reported in exposed animals. Unclear if bradypnea was present.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	No statsitics Data for mortality and terminal BW were reported adequately for independent analysis. Clinical signs data inadequate for independent analysis.
	Metric 24:	Reporting of Data	Medium	× 2	4	Quantitative mortality and body weight data. Exposure-related clinical signs reported qualitatively.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

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Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	1-d inhalation studies - rats and mice
HERO ID:	632655

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: **Animal toxicity evaluation results of NTP 1986 for 14-day inhalation studies in rats and mice on neurological/behavioral, mortality, nutrition and metabolic/adult exposure body weight outcomes**

Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)					
Data Type:	14-d inhalation studies - rats and mice - Mortality, BW, Neurological/Behavioral					
HERO ID:	632655					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	high-purity tetrachloroethylene, Dowper stabilized
	Metric 2:	Test Substance Source	High	× 1	1	Dow Chemical, lot TA03116F-01. Purity and identity analyses conducted.
	Metric 3:	Test Substance Purity	High	× 1	1	Confirmed analytically - approximately 99.9%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Chamber controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed for study type.
	Metric 6:	Randomized Allocation	Medium	× 1	2	stratified by weight then assigned to groups according to a table of random numbers (weight is a non-random component)
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Tetrachloroethylene was found to be stable for 2 weeks at 60" C (Appendix H). Tetrachloroethylene was stored at 0" C Tetrachloroethylene was vaporized at 100"- 110" C, diluted with air, and. introduced into the chambers. Detailed descriptions in Table 2 and in Appendix I.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Concentrations in the exposure chambers were monitored 8-12 times per exposure period by a Hewlett-Packard 5840A Gas Chromatograph. No deviations from protocol noted.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Only target concentrations reported for non-chronic studies., but actual exposures expected to be close to target based on 2-yr analytical values.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	14-d, 6 hr/d, 5 d/wk.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	5 dose groups plus control
	Metric 12:	Exposure Route and Method	Low	× 1	3	Inhalation, dynamic whole-body chamber. Flow rate not reported
Domain 4: Test Organism						

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Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	14-d inhalation studies - rats and mice - Mortality, BW, Neurological/Behavioral
HERO ID:	632655

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	High	× 2	2	F344/N rats and B6C3F1 mice, Charles River Breeding. 6-8 wks at study initiation. Initial body weights reported in Tables 7 and 18.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Some details of husbandry in Table 5; room conditions not reported.
	Metric 15:	Number per Group	High	× 1	1	5/sex/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Mortality, clinical signs, body weight
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation in all study groups for 14-d study.
	Metric 18:	Sampling Adequacy	High	× 1	1	5/sex/group
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Evaluated endpoints did not require blinding
	Metric 20:	Negative Control Response	High	× 1	1	Control responses reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no reported differences among the study groups in initial body weight. Food and water intake were not reported. Respiratory rate not reported, but dyspnea was reported at highest exposure in both rats and mice.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Detailed statistical tests reported for survival and tumor analysis of 2-yr study, unclear if any statistics were conducted on shorter-duration studies.. Data for mortality and terminal BW were reported adequately for independent analysis. Clinical signs data inadequate for independent analysis.
	Metric 24:	Reporting of Data	Medium	× 2	4	Quantitative mortality and body weight data. Exposure-related clinical signs reported qualitatively.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

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Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	14-d inhalation studies - rats and mice - Mortality, BW, Neurological/Behavioral
HERO ID:	632655

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 9: **Animal toxicity evaluation results of NTP 1986 for 14-day inhalation studies in rats and mice (histology) on reproductive, hematological and immune, renal, hepatic, cardiovascular, endocrine, gastrointestinal, respiratory, skin and connective tissue, thyroid outcomes**

Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	14-d inhalation studies - rats and mice - Histology
HERO ID:	632655

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	high-purity tetrachloroethylene, Dowper stabilized
	Metric 2:	Test Substance Source	High	× 1	1	Dow Chemical, lot TA03116F-01. Purity and identity analyses conducted.
	Metric 3:	Test Substance Purity	High	× 1	1	Confirmed analytically - approximately 99.9%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Chamber controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed for study type.
	Metric 6:	Randomized Allocation	Medium	× 1	2	stratified by weight then assigned to groups according to a table of random numbers (weight is a non-random component)
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Tetrachloroethylene was found to be stable for 2 weeks at 60" C (Appendix H). Tetrachloroethylene was stored at 0" C Tetrachloroethylene was vaporized at 100"- 110" C, diluted with air, and. introduced into the chambers. Detailed descriptions in Table 2 and in Appendix I.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Concentrations in the exposure chambers were monitored 8-12 times per exposure period by a Hewlett-Packard 5840A Gas Chromatograph. No deviations from protocol noted.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Only target concentrations reported for non-chronic studies., but actual exposures expected to be close to target based on 2-yr analytical values.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	14-d, 6 hr/d, 5 d/wk.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	5 dose groups plus control
	Metric 12:	Exposure Route and Method	Low	× 1	3	Inhalation, dynamic whole-body chamber. Flow rate not reported
Domain 4: Test Organism						

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Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	14-d inhalation studies - rats and mice - Histology
HERO ID:	632655

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Test Animal Characteristics	High	× 2	2	F344/N rats and B6C3F1 mice, Charles River Breeding. 6-8 wks at study initiation. Initial body weights reported in Tables 7 and 18.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Some details of husbandry in Table 5; room conditions not reported.
	Metric 15:	Number per Group	High	× 1	1	5/sex/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	comprehensive histopathology
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation in all study groups for 14-d study.
	Metric 18:	Sampling Adequacy	High	× 1	1	5/sex/group
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Evaluated endpoints did not require blinding
	Metric 20:	Negative Control Response	High	× 1	1	Control responses reported.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no reported differences among the study groups in initial body weight. Food and water intake were not reported. Respiratory rate not reported, but dyspnea was reported at highest exposure in both rats and mice.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	Detailed statistical tests reported for survival and tumor analysis of 2-yr study, unclear if any statistics were conducted on shorter-duration studies. Histo data not reported.
	Metric 24:	Reporting of Data	Unacceptable	× 2	8	Histological results not reported; no statement regarding lack of exposure-related findings.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		1.7	
Extracted			No			

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Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	14-d inhalation studies - rats and mice - Histology
HERO ID:	632655

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 =≥ 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 10: **Animal toxicity evaluation results of Boverhof et al 2013 for a 4-week inhalation (perc) study on mortality, nutrition and metabolic/adult exposure body weight, hematological and immune, hepatic, renal, and respiratory outcomes**

Study Citation:	Boverhof, D.R., Krieger, S.M., Hotchkiss, J., Stebbins, K.E., Thomas, J., Woolhiser, M.R. (2013). Assessment of the immunotoxic potential of trichloroethylene and perchloroethylene in rats following inhalation exposure Journal of Immunotoxicology, 10(3), 311-320
Data Type:	4-week inhalation (Perc)
HERO ID:	2127872

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	Medium	× 1	2	The source of the test substance was reported incompletely (a batch/lot number was not reported).
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity was acceptable (reported to be 99.98% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A concurrent negative control group (filtered air only) was used and was appropriate.
	Metric 5:	Positive Controls	High	× 1	1	A positive control group (injected with cyclophosphamide) was included in the antibody response test and was appropriate. A similar positive control was not included in the test for evaluating organ weights, histopathology, hematology, and bronchoalveolar lavage (not applicable).
	Metric 6:	Randomized Allocation	Low	× 1	3	The study authors 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	The test substance preparation and method and equipment used to generate the test substance as a vapor were reported and appropriate. The study authors did not report how the test substance was stored.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of the exposure administration were reported and exposures were administered consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported without ambiguity. Test concentrations in the chambers were analytically determined at least once per hour during the exposures and mean analytical concentrations were reported. The analytical method used to measure chamber concentrations was reported and appropriate.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration of exposure were reported and appropriate for the study and outcomes of interest.

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Study Citation: Boverhof, D.R., Krieger, S.M., Hotchkiss, J., Stebbins, K.E., Thomas, J., Woolhiser, M.R. (2013). Assessment of the immunotoxic potential of trichloroethylene and perchloroethylene in rats following inhalation exposure Journal of Immunotoxicology, 10(3), 311-320
Data Type: 4-week inhalation (Perc)
HERO ID: 2127872

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and concentration spacing were justified by the study authors (based on previous studies/animal data) 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. A dynamic whole body chamber was used and acceptable for the test substance vapor.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and age were reported and the test animals were obtained from a commercial source. Initial body weights and health status at the start of the study were not reported although the animals were certified Virus Antibody Free by the source.
	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 if differences occurred between control and exposed groups.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals per group (8 females/dose group) was less than the typical number used in studies of the same or similar type (e.g., subchronic toxicity study).
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.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported and the study used adequate sampling for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were reported.
	Metric 20:	Negative Control Response	High	× 1	1	The biological response of the negative control group was reported and acceptable.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not reported to have been evaluated in this inhalation study; however, Perc is a potential respiratory irritant.

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Study Citation:	Boverhof, D.R., Krieger, S.M., Hotchkiss, J., Stebbins, K.E., Thomas, J., Woolhiser, M.R. (2013). Assessment of the immunotoxic potential of trichloroethylene and perchloroethylene in rats following inhalation exposure Journal of Immunotoxicology, 10(3), 311-320
Data Type:	4-week inhalation (Perc)
HERO ID:	2127872

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
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	Medium	× 2	4	Data for most exposure-related findings were reported for most, but not all, outcomes by exposure group. However, some exposure-related data were not reported quantitatively (e.g., reduced body weights) and incidence data for histopathological findings were reported incompletely (only the mid- and high-concentrations; unclear if any animals were affected in the control or low-concentration groups).
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 Seo et al 2012 for a 2- to 4-wk drinking water exposure study in mice on hematological and immune outcomes**

Study Citation:	Seo, M., Kobayashi, R., Okamura, T., Ikeda, K., Satoh, M., Inagaki, N., Nagai, H., Nagase, H (2012). Enhancing effects of trichloroethylene and tetrachloroethylene on type I allergic responses in mice Journal of Toxicological Sciences, 37(2), 439-445					
Data Type:						
HERO ID:	2128339					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	
	Metric 2:	Test Substance Source	High	× 1	1	
	Metric 3:	Test Substance Purity	High	× 1	1	
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent control did not receive vehicle (DMSO) but author states that this concentration of DMSO did not have effects in preliminary experiments.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control is not required.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups. Some experiments were done on cells isolated from animals.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The storage of the chemical was not stated, but it is not known to be unstable (WI).
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	The drinking water dosing was changed every other day, not every day. The concentration was below the solubility, but the test compound is slightly volatile.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Nominal drinking water concentrations are provided and doses are presented as mean ug ingested per day by each group of 8 mice (not adjusted for body weight). Also, it is unclear if water intake varied among treatment groups. The IP dose injections and the in vitro doses were defined.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The dosing was in drinking water ad libitum, but the duration was defined.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Dose spacing was 10-100 fold.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Test substance if volatile, but drinking water was changed every other day.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Mouse strains were identified. Body weight and health status were not reported.

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Study Citation: Seo, M., Kobayashi, R., Okamura, T., Ikeda, K., Satoh, M., Inagaki, N., Nagai, H., Nagase, H (2012). Enhancing effects of trichloroethylene and tetrachloroethylene on type I allergic responses in mice Journal of Toxicological Sciences, 37(2), 439-445

Data Type:

HERO ID: 2128339

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Minimal details on husbandry conditions were provided. The dietary mix was not identified.
	Metric 15:	Number per Group	Unacceptable	× 1	4	The number of animals per study group was not reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcomes were consistent across experiments.
	Metric 18:	Sampling Adequacy	Low	× 1	3	It is not clear what the experimental unit was (i.e., whether the outcome was measured separately for each individual animal).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Outcome was not subjective. The measurements used analytical devices.
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Water intake was not reported separately for each dose group, so it is unclear whether there were differences in water intake among doses. The in vitro study and the IP study designs were better controlled.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Heath outcomes unrelated to exposure were not reported; however, no differences in health among study groups were reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Limited details regarding statistics were provided. Graphs were plotted for the results, but the numerical raw data was not provided.
	Metric 24:	Reporting of Data	High	× 2	2	
Overall Quality Determination‡			Unacceptable⋆⋆		1.8	
Extracted			Yes			

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Study Citation:	Seo, M., Kobayashi, R., Okamura, T., Ikeda, K., Satoh, M., Inagaki, N., Nagai, H., Nagase, H (2012). Enhancing effects of trichloroethylene and tetrachloroethylene on type I allergic responses in mice Journal of Toxicological Sciences, 37(2), 439-445
Data Type:	
HERO ID:	2128339

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 =≥ 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

3 Dermal Absorption

Table 12: **In vitro evaluation results for Nakai et al 1999 for dermal absorption of Perc**

Study Citation:	J. S. Nakai, P. B. Stathopulos, G. L. Campbell, I. Chu, A. Li-Muller, R. Aucoin (1999). Penetration of chloroform, trichloroethylene, and tetrachloroethylene through human skin Journal of Toxicology and Environmental Health, Part A: Current Issues, 58(3,3), 157-170
Data Type:	In vitro dermal absorption of Perc
HERO ID:	630816

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was definitively identified using established nomenclature.
	Metric 2:	Test Substance Source	High	× 1	1	Commercial source (Sigma Chemical) of radiolabeled test chemical was provided with details on specific activity.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity was not given; however, the specific activity of the 14C-radiololabeled compound was provided.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Negative controls were not necessary for this study type.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not necessary for this study type.
	Metric 6:	Assay Procedures	High	× 1	1	Methods were well described and appropriate, especially controlling for volatility.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	The preparation and storage of the radiolabeled test substance were not described.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	The concentration of the donor solution was measured each hour and replenished as required
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Specific activity was reported; additional study details were given in a previous publication.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Steady state permeability was determined following 8h exposure.
	Metric 12:	Exposure Route and Method	Not Rated	NA	NA	Determination of steady state permeability did not require multiple exposure groups; goal was to provide infinite dose exposure by replenishing the donor solution hourly.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	
Domain 4: Test Model						

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Study Citation:	J. S. Nakai, P. B. Stathopulos, G. L. Campbell, I. Chu, A. Li-Muller, R. Aucoin (1999). Penetration of chloroform, trichloroethylene, and tetrachloroethylene through human skin Journal of Toxicology and Environmental Health, Part A: Current Issues, 58(3,3), 157-170
Data Type:	In vitro dermal absorption of Perc
HERO ID:	630816

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	Test model was routinely used and source described; in vitro human skin preparation system, modified for evaluations of volatile compounds.
	Metric 15:	Number per Group	High	× 1	1	Mean Kp values estimated for 6 fresh tissue obtained from human abdomen and breast and for 5 frozen tissues for comparison. 5-6 cells/tissue.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Analysis of cumulative radiolabel in receptor fluid by scintillation counting.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistently assessed across tissues.
	Metric 18:	Sampling Adequacy	High	× 2	2	5-6 cells per tissue; 5-6 tissues used.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were assessed.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Both breast and abdominal skin samples were obtained from different donors.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	Analysis of radiolabel reduces the possibility of confounding unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Methods for calculating cumulative permeation, chemical flux and permeability coefficient were clearly described.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Scoring and evaluation criteria are not applicable to this method.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity is not relevant to the test method.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for individual tissue samples as well as mean +- SD for Kp.
Overall Quality Determination‡			High		1.2	
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

4 Subchronic (30-90 days)

Table 13: **Animal toxicity evaluation results of E. I. Dupont De Nemours 1941 for a 10 week inhalation study in dogs on neurological/behavior, cardiovascular, hematological and immune outcomes**

Study Citation:	Dupont (E I Dupont De Nemours & Co) (1941). Initial submission: The toxicity of perchloroethylene with cover letter dated 10/15/92
Data Type:	10 week inhalation study in dogs
HERO ID:	4214432

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	Medium	× 2	4	Test substance identified by unambiguous name and molecular formula, but without certification or validation of identity.
	Metric 2:	Test Substance Source	Low	× 1	3	Test substance source was not reported, and given the age of the study, it was probably not obtained from a manufacturer.
	Metric 3:	Test Substance Purity	Low	× 1	3	Test substance purity/grade not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	A concurrent negative control group was not included; animals served as their own controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	positive control not typical for this study type.
	Metric 6:	Randomized Allocation	Unacceptable	× 1	4	Animals were not allocated to groups; rather, health outcomes assessed before and after exposure in all animals
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	No information on test substance preparation or storage, or methods for atmosphere generation, was presented.
	Metric 8:	Consistency of Exposure Administration	Unacceptable	× 1	4	There were no details provided to enable assessment of consistency, except that exposure concentrations were increased over the course of the exposure period.
	Metric 9:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	Exposure concentrations were reported inconsistently within the study; the methods section reports concentrations that differ from those in the results sections.. Study reported exposure concentrations without any indication of how these were estimated or measured. There is no indication that exposure concentrations were verified analytically.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Dogs were exposed 6 hr/d, 5 d/wk for 10 weeks and Guinea Pigs were exposed for two weeks (No exposure detail reported)

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Study Citation: Dupont (E I Dupont De Nemours & Co) (1941). Initial submission: The toxicity of perchloroethylene with cover letter dated 10/15/92
Data Type: 10 week inhalation study in dogs
HERO ID: 4214432

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 11:	Number of Exposure Groups and Dose Spacing	Unacceptable	× 1	4	Only one group of animals was included; these animals were exposed to increasing concentrations over time, and effects compared with pre-exposure conditions.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	There is no description of the inhalation chamber used
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	Test animal source, strain, and sex were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	No information on animal husbandry was provided.
	Metric 15:	Number per Group	Low	× 1	3	Four animals were exposed, and served as their own controls.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Unacceptable	× 2	8	The outcome assessment methodologies were not reported, and the outcomes assessed were not sensitive (oxygen content of blood, electrocardiography, some hematology endpoints, and gross pathology)
	Metric 17:	Consistency of Outcome Assessment	Unacceptable	× 1	4	Outcome assessments were not adequately reported for meaningful interpretation of results.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Information was not adequate to evaluate sampling adequacy, but it appears that all animals were evaluated for all endpoints.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Most outcomes were not subjective.
	Metric 20:	Negative Control Response	Unacceptable	× 1	4	There was no control group; dogs served as their own controls.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No information on potential confounding factors was reported. Initial body weight and food and water intake were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Data on attrition or health outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Unacceptable	× 1	4	Statistical analysis was not performed, and reported data were not adequate to enable independent statistical analysis.
	Metric 24:	Reporting of Data	Unacceptable	× 2	8	Most data were reported qualitatively and without clear reference to the pre-exposure response.
Overall Quality Determination[‡]			Unacceptable[⋆⋆]		3.4	

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Study Citation:	Dupont (E I Dupont De Nemours & Co) (1941). Initial submission: The toxicity of perchloroethylene with cover letter dated 10/15/92
Data Type:	10 week inhalation study in dogs
HERO ID:	4214432

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 $=\geq 1$ 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 14: **Animal toxicity evaluation results of Natl Institute of Health 1977 for a 6-week oral (rats and mice) study on mortality and metabolic/adult exposure body weight outcomes**

Study Citation:	NIH (National Institutes of Health) (1977). Bioassay of tetrachloroethylene for possible carcinogenicity					
Data Type:	6-week oral (rats and mice)					
HERO ID:	4214470					
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	Medium	× 1	2	The source of the test substance was reported, including manufacturer. A lot/batch number was not reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity was reported by the manufacturer (at least 99%). The study report also stated that gas-liquid chromatography showed the major component consisting of over 99% of the total peak area, with a minor impurity present, which was not identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate concurrent control group (vehicle control administered corn oil only).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control is not indicated for the study type.
	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	The test substance preparation and storage conditions were reported but there were minor limitations in the test substance preparation. The test substance was prepared weekly, sealed, and stored at 34 degrees F, which the study authors noted were considered conditions that would allow test substance to remain stable for 10 days. However, no report of stability in the vehicle (corn oil), or of PERC in the prepared solutions, was reported.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were not fully reported (volume administered by gavage was not reported). However, reported information indicated that exposures were administered consistently across study groups.

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Study Citation: NIH (National Institutes of Health) (1977). Bioassay of tetrachloroethylene for possible carcinogenicity
Data Type: 6-week oral (rats and mice)
HERO ID: 4214470

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Initial administered doses were reported; however, dose levels were raised and/or lowered during the study in both rats and mice based on clinical signs and there is some ambiguity in the actual dose levels after adjustment and the exact days during the study when doses were raised and/or lowered (only reported in weeks). For example, for rats, the study authors stated that the low doses were adjusted accordingly, so that they consistently remained one-half of the high dose but actual adjusted dose levels were not reported.
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Exposure frequency (5 consecutive d/wk) was reported and acceptable. However, the exposure duration was shorter than studies of similar type (i.e., 2 years for carcinogenicity studies is typical for rodents) and was not justified by the study authors. In this study, animals were dosed for 78 weeks followed by an observation period of 32 weeks in rats and 12 weeks in mice.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups and spacing were considered adequate to address the purpose of the study. However, the highest doses produced a high rate of early mortality in both rats and mice, which the study authors noted may indicate that the optimum dose was exceeded in both species.
	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	Medium	× 2	4	The test animal source, species, strain, sex, age, and starting body weight were reported. The test animal (species, strain, sex, life-stage, source) was appropriate for the evaluation of the specific outcome(s) of interest.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Due to starting the vehicle control rats and mice earlier than animals of other groups, and housing of vehicle control rats and a different room than other rats, there may have been some differences in husbandry / exposure conditions.
	Metric 15:	Number per Group	High	× 1	1	The number per group was acceptable (5/sex/group) for the 6-week, range-finding study
Domain 5: Outcome Assessment						

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Study Citation: NIH (National Institutes of Health) (1977). Bioassay of tetrachloroethylene for possible carcinogenicity
Data Type: 6-week oral (rats and mice)
HERO ID: 4214470

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	The outcome assessment methodology was only briefly reported. For example, it was not reported how often body weights were determined during the 6-week dosing period and 2-week observation period. Additionally, the only endpoints evaluated were grossly observable endpoints, including clinical signs and mortality.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Details of the outcome assessment protocol were not reported and these deficiencies are likely to have a substantial impact on results.
	Metric 18:	Sampling Adequacy	Low	× 1	3	Details regarding sampling of outcomes were not reported and this deficiency is likely to have a substantial impact on results.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were reported.
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables in test design or procedures were reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	The statistical methods were clearly described by the study authors and were appropriate for datasets.
	Metric 24:	Reporting of Data	Low	× 2	6	Data were reported incompletely. Body weights were reported in figures and changes in body weight gain were reported in percentages in the text.
Overall Quality Determination‡			~~Medium~~ ⟶ Low§		~~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 =≥ 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 reviewer downgraded this study's overall quality rating based on limited reporting of outcome assessment methodology and protocol and limited reporting of data. Although a score was calculated, it is not presented here because the final rating was changed based on professional judgement."

Table 15: **Animal toxicity evaluation results of Buben et al 1985 for a 6 week gavage study of perc in mice study on hepatic outcomes**

Study Citation:	Buben, JA; O'Flaherty, EJ (1985). Delineation of the role of metabolism in the hepatotoxicity of trichloroethylene and perchloroethylene: A dose-effect study Toxicology and Applied Pharmacology, 78(1), 105-122
Data Type:	6 week gavage study of Perc in mice
HERO ID:	65239

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name
	Metric 2:	Test Substance Source	High	× 1	1	Test substance obtained commercially
	Metric 3:	Test Substance Purity	High	× 1	1	Perc reported to have purity >99%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Sham-treated controls received corn oil vehicle.
	Metric 5:	Positive Controls	Not Rated	NA	NA	
	Metric 6:	Randomized Allocation	High	× 1	1	Study reports random allocation to study groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation method was reported and appropriate (prepared fresh 2-3x/wk); stability of test material in vehicle was either not evaluated or not reported,. but not expected to be of concern given the frequency of preparation.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Details of administration (e.g., time of day) were not reported; no dosing errors were noted.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose volumes were adjusted based on individual animal body weights obtained 3x/week.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Animals were dosed 5 days/week for 6 weeks. The duration was sufficient to induce the effects of interest (hepatotoxicity).
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Study used 7 exposure groups plus control; overall range of doses was 100-fold; high dose was adequate to identify effect. The lowest Perc dose of 20 mg/kg may be a NOAEL, but histopathology was only evaluated at 200mg/kg and 1000 mg/kg (effects seen at both) so it is difficult to determine the NOAEL.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route and method were appropriate for the study type and test material.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal source, strain, sex, and age were reported. The ages of mice at study initiation varied between 3 and 5 months; however, as mice are adult at these ages, the age range is not expected to influence hepatotoxicity.

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Study Citation:	Buben, JA; O'Flaherty, EJ (1985). Delineation of the role of metabolism in the hepatotoxicity of trichloroethylene and perchloroethylene: A dose-effect study Toxicology and Applied Pharmacology, 78(1), 105-122
Data Type:	6 week gavage study of Perc in mice
HERO ID:	65239

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	Temperature and light-dark cycle, and housing conditions were reported and appropriate, but humidity was not reported.
	Metric 15:	Number per Group	High	× 1	1	Test animal source, strain, sex, and age were reported. The ages of mice at study initiation varied between 3 and 5 months; however, as mice are adult at these ages, the age range is not expected to influence hepatotoxicity. A two-month spread in ages is not a concern, especially since animals were randomly allocated.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Study focused on hepatotoxicity based on organ weight, liver G6P activity and triglycerides, serum ALT, and histopathology.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Study did not report any inconsistencies in execution of outcome assessments. Histopathy was only reported in two dose groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Incomplete information was provided on sampling adequacy across endpoints. HIstopathology examinations were performed on controls, high dose animals, and on animals of one intermediate dose group.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	
	Metric 20:	Negative Control Response	High	× 1	1	Responses of negative control group were adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	Study did not report any potential differences among study groups that might influence the assessment.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	There were no reported differences among groups unrelated to exposure
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were reported and appeared to be appropriate.
	Metric 24:	Reporting of Data	Medium	× 2	4	Histopathology results were reported semiquantitatively (incidences not reported); no statistical analysis of incidences was performed, and the available data are not adequate to perform independent statistical analysis. Data was quantitatively reported for all outcomes other than histopathy at all dose groups.
Overall Quality Determination[‡]			~~High~~ ⟶ Medium[§]		~~1.3~~	

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Study Citation:	Buben, JA; O'Flaherty, EJ (1985). Delineation of the role of metabolism in the hepatotoxicity of trichloroethylene and perchloroethylene: A dose-effect study Toxicology and Applied Pharmacology, 78(1), 105-122
Data Type:	6 week gavage study of Perc in mice
HERO ID:	65239

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.

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

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: "HIstopathology examinations were performed in control, 200 and 1000 mg/kg dose groups, and lesions were seen in both exposed groups. Although there were lower dose groups in which no changes in other parameters were observed, it would be difficult to identify a NOAEL in the absence of confirmatory histopathology results for the lower dose groups."

5 Chronic (>90 days)

Table 16: **Animal toxicity evaluation results of Jisa et al 1993 for a cancer bioassay study on cancer; nutrition and metabolic/adult exposure body weight outcomes**

Study Citation:	JISA (1993). Carcinogenicity study of tetrachloroethylene by inhalation in rats and mice
Data Type:	Cancer bioassay
HERO ID:	630653

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Structural formula, CASRN, physiochemical properties were provided
	Metric 2:	Test Substance Source	High	× 1	1	Source and lot numbers provided; identity verified by mass spec and infrared absorption spectrum of each lot
	Metric 3:	Test Substance Purity	High	× 1	1	Purity such that effects likely due to test subsstance
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 control animals were not required for this study
	Metric 6:	Randomized Allocation	Medium	× 1	2	Animals assigned to each treatment group by grouping method (optimal stratification system).
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Method of generating vapor and storage was described in detail and appropriate
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Nominal and analytical concentrations were reported, tetrachloroethylene concentration inside the inhalation chamber was determined before exposure started and then every 15 minutes until exposure was completed using GC.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The frequency and duration were reported and appropriate
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The rationale for the exposure concentrations and number of groups were reported.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were adequate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, age, health, sex, starting body weight provided for both rats and mice
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Conditions were reported and the same across groups.

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Study Citation: JISA (1993). Carcinogenicity study of tetrachloroethylene by inhalation in rats and mice
Data Type: Cancer bioassay
HERO ID: 630653

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number was reported and appropriate. 50/sex/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcomes of interest
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes assess consistently across groups
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was adequate for the outcomes
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required
	Metric 20:	Negative Control Response	High	× 1	1	Negative responses were adequate
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variable reported
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No confounding variables reported
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical methods were appropriate
	Metric 24:	Reporting of Data	Medium	× 2	4	Data for non-cancer endpoints summarized in text, but specific details not 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 $=\geq 1$ 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 Maltoni et al 1986 for a 2-yr carcinogenicity bioassay - oral - rats study on cancer outcomes**

Study Citation:	Maltoni, C; Cotti, G (1986). Results of long-term carcinogenicity bioassays of tetrachloroethylene on Sprague-Dawley rats administered by ingestion Acta Oncologica (Italy), 7(1), 11-26					
Data Type:	2-year carcinogenicity bioassay - oral - rats					
HERO ID:	630745					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as TTCE (tetrachloroethylene) Note: This study has been listed under TCE, but the chemical compound test is Tetrachloroethyele (Perc)
	Metric 2:	Test Substance Source	Low	× 1	3	Omitted details on the source of the test substance
	Metric 3:	Test Substance Purity	Medium	× 1	2	several impurities have been reported in the test chemical; carbon tetrachloride (53 ppm), 1,1,2-trichloroethane (11 ppm), and asymmetrical tetrachloroethane (20 ppm). They may not have substantial impact on the results
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Extra-virgin olive oil was used as a vehicle control
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not applicable for this study type
	Metric 6:	Randomized Allocation	Medium	× 1	2	random allocation was noted as "divided into groups by litter distribution".
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Some preparation information was reported. No storage information was provided
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	The animals were exposed once daily, 4-5 days weekly, for 104 weeks
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	The dose tested was reported (500 mg/kg/bw), however, only one dose was tested
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	daily (4-5 days per week) for 104 weeks
	Metric 11:	Number of Exposure Groups and Dose Spacing	Unacceptable	× 1	4	Only one dose tested; the single dose was not justified by the study authors. CK: Also, according to PECO, at least two dose groups are needed
	Metric 12:	Exposure Route and Method	High	× 1	1	gavage
Domain 4: Test Organism						

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Study Citation:	Maltoni, C; Cotti, G (1986). Results of long-term carcinogenicity bioassays of tetrachloroethylene on Sprague-Dawley rats administered by ingestion Acta Oncologica (Italy), 7(1), 11-26
Data Type:	2-year carcinogenicity bioassay - oral - rats
HERO ID:	630745

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The source of test animals was unclear; animals were noted to be the same breed used for bioassays in the experimental laboratories of the author's institute; unclear the impact on results. strain, sex and age were reported. Animals were examined throughout the study.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not sufficiently reported to evaluate if husbandry was adequate. Only temperature was reported; humidity and light-dark cycle were not reported; unclear the impact on results.
	Metric 15:	Number per Group	High	× 1	1	50/sex for control group; 40/sex for treatment group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	assessment made for each treated and control animal
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not rated/applicable; initial histopathology evaluation
	Metric 20:	Negative Control Response	Medium	× 1	2	There was a slightly higher number of tumors in control rats than in treated groups.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No notable confounding variables
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Low	× 1	3	Statistical analysis was not described clearly
	Metric 24:	Reporting of Data	High	× 2	2	average body weight, tumors at various sites were reported
Overall Quality Determination‡			Unacceptable⋆⋆		1.6	
Extracted			Yes			

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Study Citation:	Maltoni, C; Cotti, G (1986). Results of long-term carcinogenicity bioassays of tetrachloroethylene on Sprague-Dawley rats administered by ingestion Acta Oncologica (Italy), 7(1), 11-26
Data Type:	2-year carcinogenicity bioassay - oral - rats
HERO ID:	630745

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 =≥ 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 18: **Animal toxicity evaluation results of NTP 1986 for 2-year cancer biossay, inhalation studies in rats and mice on cancer, reproductive, hematological and immune, neurological/behavior, renal, hepatic, cardiovascular, endocrine, gastrointestinal, mortality, nutrition and metabolic/adult exposure body weight, respiratory, skin and connective tissues, thyroid outcomes**

Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	2-yr cancer biossay, inhalation - rats and mice
HERO ID:	632655

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	high-purity tetrachloroethylene, Dowper stabilized
	Metric 2:	Test Substance Source	High	× 1	1	Dow Chemical, lots TA03116F-01 and TA08190D. Purity and identity analyses conducted.
	Metric 3:	Test Substance Purity	High	× 1	1	Confirmed analytically for both lots - approximately 99.9%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Chamber controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed for study type.
	Metric 6:	Randomized Allocation	High	× 1	1	computer generated tables of random numbers.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Tetrachloroethylene was found to be stable for 2 weeks at 60" C (Appendix H). Tetrachloroethylene was stored at 0" C Tetrachloroethylene was vaporized at 100"- 110" C, diluted with air, and. introduced into the chambers. Detailed descriptions in Table 2 and in Appendix I.
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Concentrations in the exposure chambers were monitored 8-12 times per exposure period by a Hewlett-Packard 5840A Gas Chromatograph. On one occasion (September 13, 1982) in the 2-year studies, the concentration in the 400- ppm chamber was 800 ppm for 12 minutes and 2,400 ppm for 48 minutes. Animals were therefore not exposed at all on September 14, 1982
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and analytical exposure levels reported for 2 yr study in rats and mice only. Mean analytical concentrations (99.5, 201, 403 ppm) very close to target (100, 200, 400 ppm).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	2-yr, 6 hr/d, 5 d/wk.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	2 dose groups plus control

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Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	2-yr cancer biossay, inhalation - rats and mice
HERO ID:	632655

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	Inhalation, dynamic whole-body chamber. Flow rate not reported For the chemistry data, all of the available records concerning receipt, initial analysis, and stability testing by Midwest Research Instiitute (MRI) were examined. In addition, records pertaining to receipt, bulk chemical analysis, generation of chamber concentrations, exposure chamber monitoring, and gas chromatographic calibration by the study laboratory were examined.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	F344/N rats and B6C3F1 mice, Charles River Breeding. 8-9 wks at study initiation. Initial BW reported in Tables 10 and 21, respectively.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Details of husbandry in Table 5
	Metric 15:	Number per Group	High	× 1	1	49-50/sex/group per species
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Mortality, clinical signs, body weight, comprehensive histopathology
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation in all study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	49-50/sex/group
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	For histo - Slides/tissues are generally not evaluated in a blind fashion (i.e., without knowledge of dose group) unless the lesions in question are subtle or unless there is an inconsistent diagnosis of lesions by the laboratory pathologist and pathology work group. Evaluated endpoints did not require blinding.
	Metric 20:	Negative Control Response	High	× 1	1	Control responses reported. Historical incidences of tumors in control animals also reported.
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 in initial body weight. Food and water intake were not reported. Respiratory rate was not specifically mentioned, but no exposure-related clinical signs were reported. While there is no evidence of bradypnea. Animal temperature should be measured to rule out bradypnea.

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Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	2-yr cancer biossay, inhalation - rats and mice
HERO ID:	632655

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Detailed statistical tests reported for survival and tumor analysis. Appendices C and D contain non-neoplastic data reporting sufficient for statistical analysis. Body weight data not adequate for independent analysis (no variance data)
	Metric 24:	Reporting of Data	Medium	× 2	4	quantitative mortality, body weight, nonneoplastic, and neoplastic data. Clinical signs data not 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 19: **Animal toxicity evaluation results of NTP 1986 for 13-week inhalation studies in rats and mice on reproductive, hematological and immune, neurological/behavior, renal, hepatic, cardiovascular, endocrine, gastrointestinal, mortality, nutrition and metabolic/adult exposure body weight, respiratory, skin and connective tissue, and thyroid outcomes**

Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	13-wk inhalation studies - rats and mice
HERO ID:	632655

Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	high-purity tetrachloroethylene, Dowper stabilized
	Metric 2:	Test Substance Source	High	× 1	1	Dow Chemical, lot TA03116F-01. Purity and identity analyses conducted.
	Metric 3:	Test Substance Purity	High	× 1	1	Confirmed analytically - approximately 99.9%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Chamber controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed for study type.
	Metric 6:	Randomized Allocation	High	× 1	1	computer generated tables of random numbers
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Tetrachloroethylene was found to be stable for 2 weeks at 60" C (Appendix H). Tetrachloroethylene was stored at 0" C Tetrachloroethylene was vaporized at 100"- 110" C, diluted with air, and. introduced into the chambers. Detailed descriptions in Table 2 and in Appendix I.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Concentrations in the exposure chambers were monitored 8-12 times per exposure period by a Hewlett-Packard 5840A Gas Chromatograph. No deviations from protocol noted.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Only target concentrations reported for non-chronic studies., but actual exposures expected to be close to target based on 2-yr analytical values.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	13-wk, 6 hr/d, 5 d/wk.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	5 dose groups plus control
	Metric 12:	Exposure Route and Method	Low	× 1	3	Inhalation, dynamic whole-body chamber. Flow rate not reported
Domain 4: Test Organism						

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Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	13-wk inhalation studies - rats and mice
HERO ID:	632655

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Test Animal Characteristics	High	× 2	2	F344/N rats and B6C3F1 mice, Charles River Breeding. 7-9 wks at study initiation. Initial body weights reported in Tables 8, and 19.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Details of husbandry in Table 5
	Metric 15:	Number per Group	High	× 1	1	10/sex/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Mortality, clinical signs, body weight, comprehensive histopathology
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The majority of organs/tissues were only evaluated in control and high-dose groups. Organs with exposure-related findings were evaluated in lower-dose groups as needed.
	Metric 18:	Sampling Adequacy	High	× 1	1	10/sex/group
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Evaluated endpoints did not require blinding
	Metric 20:	Negative Control Response	High	× 1	1	Control responses reported.
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 in initial body weight. Food and water intake were not reported. Respiratory rate was not specifically mentioned, but no exposure-related clinical signs were reported. While there is no evidence of bradypnea. Animal temperature should be measured to rule out bradypnea.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Medium	× 1	2	Detailed statistical tests reported for survival and tumor analysis of 2-yr study, unclear if any statistics were conducted on shorter-duration studies.. Data for mortality, terminal BW, liver and lung histo findings (rat) and liver and kidney findings (mouse) were adequately reported for independent analysis.

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Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	13-wk inhalation studies - rats and mice
HERO ID:	632655

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	Low	× 2	6	Quantitative mortality, body weight, and exposure-related nonneoplastic findings (lung and liver in rats, liver and kidney in mice). Histological results from other organs not reported; assumed to be no exposure-related findings.. Exposure-related clinical signs reported qualitatively in mice.
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 20: **Animal toxicity evaluation results of Dow et al 1978 for a 12 month inhalation study in rats, with lifetime observation on renal, hepatic, nutrition and metabolic/adult exposure body weight, hematological and immune outcomes**

Study Citation:	Dow Chemical Company (1978). Results of a long-term inhalation toxicity study on rats of a perchloroethylene (tetrachloroethylene) formulation
Data Type:	12 month inhalation study in rats, with lifetime observation
HERO ID:	4214237

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name and CASRN
	Metric 2:	Test Substance Source	High	× 1	1	Test substance was identified by lot number and verified analytically, with results presented.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was not reported explicitly, but based on GC results and reported percentages of contaminants, test substance was >99% (vol%) perc (impurities comprised 63 ppm vol %)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Negative controls were not sham-exposed, but rather held in the room where exposed animals were housed when not in exposure chambers.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for this study type
	Metric 6:	Randomized Allocation	High	× 1	1	Study reported random allocation
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Method of vapor generation was described in detail and appropriate (dynamic airflow); however, there was no diagram of the chamber, so it is unclear whether vertical mixing was adequate (Perc vapor is much heavier than air) and/or whether analytical measurements were in the animals' breathing zones.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Control animals were not sham-exposed. Authors report that exposures during first 5 months ran at the same time in both exposed groups, but thereafter they ran at different times of day (low dose in morning and high dose in evening) using the same exposure chamber. Finally, the high dose group was accidentally exposed to concentrations of 1500 ppm for 3 days during the first week.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Concentrations were measured using infrared spectrophotometry and analytical results were reported. Mean analytical values were within 10% of nominal. Analytical method was less than ideal, and it is unclear whether the measurements were in the animals' breathing zones. Time to achieve desired exposure concentration in the chambers was not reported,.

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Study Citation:	Dow Chemical Company (1978). Results of a long-term inhalation toxicity study on rats of a perchloroethylene (tetrachloroethylene) formulation
Data Type:	12 month inhalation study in rats, with lifetime observation
HERO ID:	4214237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Frequency (6 hr/d, 5 d/wk) and duration (12 mo) of exposure were reported and appropriate for non-cancer endpoints.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Two exposure concentrations differing 2-fold were tested; these were selected based on multiples of the maximum permissible excursion concentration from ACGIH. Little to no toxicity was reported, suggesting that the high concentration may not have been high enough.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Route and method were reported and appropriate (dynamic whole body chamber was used for vapor that may condense.)
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species, strain, sex, age, source, and body weight were reported; however, authors did not report acclimation or pathogen testing/health status prior to study initiation.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions (temperature, humidity, light-dark cycle, housing) were not reported.
	Metric 15:	Number per Group	High	× 1	1	Exposed groups consisted of 96/sex and controls consisted of 192/sex.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Unacceptable	× 2	8	Nearly all evaluations took place 12 to 19 months after the end of exposure. Hematology (with the exception of a small number of animals evaluated earlier), clinical chemistry, and urinalysis evaluations were performed 12 months after exposure ended or at terminal necropsy up to 19 months after the end of exposure. Except for groups of 3 rats/sex/exposure, organ weight and pathology assessments occurred at death/moribund sacrifice or at study termination 19 months after exposure ended. Hematology and clinical chemistry methods were not reported.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Outcome assessment was performed consistently across groups. Apart from the unexplained loss of a few rats per group, which was evaluated under health outcomes unrelated to exposure, no inconsistencies in the execution were noted.

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Study Citation:	Dow Chemical Company (1978). Results of a long-term inhalation toxicity study on rats of a perchloroethylene (tetrachloroethylene) formulation
Data Type:	12 month inhalation study in rats, with lifetime observation
HERO ID:	4214237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	Unacceptable	× 1	4	Sampling of endpoints at the end of exposure was not adequate; only 3/sex/group were sacrificed for organ weights and histopathology at the end of the 12 month exposure. This number is too small to discern subtle differences.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not reported for subjective outcomes consisting of cageside observations. Other endpoints were not subjective and/or blinding is not typical.
	Metric 20:	Negative Control Response	High	× 1	1	Control responses were reported and appeared to be adequate and without excessive variability.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No information on respiratory rates or indications of reflex bradypnea was reported. Food and water intake during the study were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Study authors reported unexplained discrepancies between initial animal numbers and final animal numbers (instead of 96/sex/exposure group and 192/sex controls, 91 to 94/sex/exposure group and 189/sex controls were accounted for). However, the remaining numbers were sufficient to observe an effect and the attrition appeared to be essentially consistent across groups so this discrepancy was not considered unacceptable.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analyses were performed and described, and appropriate to the endpoints.
	Metric 24:	Reporting of Data	High	× 2	2	All data were reported with measures of variability and numbers evaluated.
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 =≥ 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 Dow et al 1978 for a 12 month inhalation study in rats, with lifetime observation (cancer) on cancer outcomes**

Study Citation:	Dow Chemical Company (1978). Results of a long-term inhalation toxicity study on rats of a perchloroethylene (tetrachloroethylene) formulation
Data Type:	12 month inhalation study in rats, with lifetime observation (cancer)
HERO ID:	4214237

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name and CASRN
	Metric 2:	Test Substance Source	High	× 1	1	Test substance was identified by lot number and verified analytically, with results presented.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was not reported explicitly, but based on GC results and reported percentages of contaminants, test substance was >99% (vol%) perc (impurities comprised 63 ppm vol %)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Negative controls were not sham-exposed, but rather held in the room where exposed animals were housed when not in exposure chambers.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for this study type
	Metric 6:	Randomized Allocation	High	× 1	1	Study reported random allocation
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Method of vapor generation was described in detail and appropriate (dynamic airflow); however, there was no diagram of the chamber, so it is unclear whether vertical mixing was adequate (Perc vapor is much heavier than air) and/or whether analytical measurements were in the animals' breathing zones.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Control animals were not sham-exposed. Authors report that exposures during first 5 months ran at the same time in both exposed groups, but thereafter they ran at different times of day (low dose in morning and high dose in evening) using the same exposure chamber. Finally, the high dose group was accidentally exposed to concentrations of 1500 ppm for 3 days during the first week.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Concentrations were measured using infrared spectrophotometry and analytical results were reported. Mean analytical values were within 10% of nominal. Analytical method was less than ideal, and it is unclear whether the measurements were in the animals' breathing zones. Time to achieve desired exposure concentration in the chambers was not reported,.

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Study Citation: Dow Chemical Company (1978). Results of a long-term inhalation toxicity study on rats of a perchloroethylene (tetrachloroethylene) formulation
Data Type: 12 month inhalation study in rats, with lifetime observation (cancer)
HERO ID: 4214237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	Low	× 1	3	Duration (12 mo) of exposure is not considered adequate for cancer endpoints.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Two exposure concentrations differing 2-fold were tested; these were selected based on multiples of the maximum permissible excursion concentration from ACGIH. Little to no toxicity was reported, suggesting that the high concentration may not have been high enough.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Route and method were reported and appropriate (dynamic whole body chamber was used for vapor that may condense.)
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species, strain, sex, age, source, and body weight were reported; however, authors did not report acclimation or pathogen testing/health status prior to study initiation.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions (temperature, humidity, light-dark cycle, housing) were not reported.
	Metric 15:	Number per Group	High	× 1	1	Exposed groups consisted of 96/sex and controls consisted of 192/sex.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Unacceptable	× 2	8	Except for groups of 3 rats/sex/exposure, histopathology assessments occurred at death/moribund sacrifice or at study termination 19 months after exposure ended. This very long postexposure observation period may have resulted in tumor regression.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Outcome assessment was performed consistently across groups. Apart from the unexplained loss of a few rats per group, which was evaluated under health outcomes unrelated to exposure, no inconsistencies in the execution were noted.
	Metric 18:	Sampling Adequacy	Unacceptable	× 1	4	Sampling of endpoints at the end of exposure was not adequate; only 3/sex/group were sacrificed for histopathology at the end of the 12 month exposure. This number is too small to discern differences in tumor incidences.
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding is not typical for initial histopathology review.

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Study Citation:	Dow Chemical Company (1978). Results of a long-term inhalation toxicity study on rats of a perchloroethylene (tetrachloroethylene) formulation
Data Type:	12 month inhalation study in rats, with lifetime observation (cancer)
HERO ID:	4214237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Negative Control Response	High	× 1	1	Control responses were reported and appeared to be adequate and without excessive variability.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No information on respiratory rates or indications of reflex bradypnea was reported. Food and water intake during the study were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Study authors reported unexplained discrepancies between initial animal numbers and final animal numbers (instead of 96/sex/exposure group and 192/sex controls, 91 to 94/sex/exposure group and 189/sex controls were accounted for). However, the remaining numbers were sufficient to observe an effect and the attrition appeared to be essentially consistent across groups so this discrepancy was not considered unacceptable.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analyses were performed and described, and appropriate to the endpoints.
	Metric 24:	Reporting of Data	High	× 2	2	Tumor incidences were reported with numbers of animals evaluated for each organ and timepoint..
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 22: **Animal toxicity evaluation results of Natl Institute of Health 1977 for a 78-week cancer bioassay (rats and mice) study on cancer, mortality, respiratory, hepatic, renal, thyroid, cardiovascular, neurological/behavior, nutrition and metabolic/adult exposure body weight, hematological and immune, skin and connective tissue, and gastrointestinal outcomes**

Study Citation:	NIH (National Institutes of Health) (1977). Bioassay of tetrachloroethylene for possible carcinogenicity
Data Type:	78-week cancer bioassay (rats and mice)
HERO ID:	4214470

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	Medium	× 1	2	The source of the test substance was reported, including manufacturer. A lot/batch number was not reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity was reported by the manufacturer (at least 99%). The study report also stated that gas-liquid chromatography showed the major component consisting of over 99% of the total peak area, with a minor impurity present, which was not identified.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using an appropriate concurrent control group (vehicle control and untreated control groups.)
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive control is not indicated for the study type.
	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	The test substance preparation and storage conditions were reported but there were minor limitations in the test substance preparation. The test substance was prepared weekly, sealed, and stored at 34 degrees F, which the study authors noted were considered conditions that would allow test substance to remain stable for 10 days. However, no report of stability in the vehicle (corn oil), or of PERC in the prepared solutions, was reported.
	Metric 8:	Consistency of Exposure Administration	Low	× 1	3	Details of exposure administration were not fully reported (volume administered by gavage was not reported).

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Study Citation:	NIH (National Institutes of Health) (1977). Bioassay of tetrachloroethylene for possible carcinogenicity
Data Type:	78-week cancer bioassay (rats and mice)
HERO ID:	4214470

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Initial administered doses were reported; however, dose levels were raised and/or lowered during the study in both rats and mice based on clinical signs and there is some ambiguity in the actual dose levels after adjustment and the exact days during the study when doses were raised and/or lowered (only reported in weeks). For example, for rats, the study authors stated that the low doses were adjusted accordingly, so that they consistently remained one-half of the high dose but actual adjusted dose levels were not reported (p. 11 of the study report).
	Metric 10:	Exposure Frequency and Duration	Medium	× 1	2	Exposure frequency (5 consecutive d/wk) was reported and acceptable. However, the exposure duration was shorter than studies of similar type (i.e., 2 years for carcinogenicity studies is typical for rodents) and was not justified by the study authors. In this study, animals were dosed for 78 weeks followed by an observation period of 32 weeks in rats and 12 weeks in mice.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	The number of exposure groups was considered adequate for the purpose of the study. However, the highest doses produced a high rate of early mortality in both rats and mice, which the study authors noted may indicate that the optimum dose was exceeded in both species.
	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	Medium	× 2	4	The test animal source, species, strain, sex, age, and starting body weight were reported. However, health status at the beginning of the study was not reported.

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Study Citation: NIH (National Institutes of Health) (1977). Bioassay of tetrachloroethylene for possible carcinogenicity
Data Type: 78-week cancer bioassay (rats and mice)
HERO ID: 4214470

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	The study authors stated that housing rooms were maintained in a temperature range of 20 to 24 deg C, a relative humidity of 45 to 55%, with a 12-hour light cycle and 12 complete changes of room air per hour. However, some differences between PERC-treated / untreated control animals and the vehicle control animals were reported, which included that PERC-treated / untreated control rats were housed in one room while the vehicle control rats were housed in another room. The study authors also reported that the vehicle control rats were approximately 4 weeks older than rats in the PERC-treated and untreated control groups and, therefore, were started on the test 4 weeks earlier. Similarly, vehicle control mice were approximately 2 weeks older than mice in the other groups and, therefore, were started on the test earlier. Due to starting the vehicle control rats and mice earlier than animals of other groups, and housing of vehicle control rats and a different room than other rats, there may have been some differences in husbandry / exposure conditions.
	Metric 15:	Number per Group	Medium	× 1	2	The number of animals in the PERC-treated groups (50/sex/group) was reported, appropriate for the study type and outcome analysis, and consistent with studies of the same or similar type; however, the number of animals in each of the two control groups (vehicle and untreated each had 20/sex/group) was lower than the typical number used in studies of the same or similar type.
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.
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported and the study used adequate sampling for the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	No subjective outcomes were reported and histopathology examinations were not described as a re-evaluation
	Metric 20:	Negative Control Response	High	× 1	1	The biological responses of the negative control groups were adequate.

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Study Citation:	NIH (National Institutes of Health) (1977). Bioassay of tetrachloroethylene for possible carcinogenicity
Data Type:	78-week cancer bioassay (rats and mice)
HERO ID:	4214470

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	Medium	× 2	4	There were minor uncertainties regarding biological responses of the negative control. For example, in mice, while no appreciable differences in body weight gain were observed between PERC-treated and untreated mice, PERC-treated male mice gained less than vehicle control animals after the first three months and PERC-treated female mice gained less than vehicle control animals during the second year of the bioassay. These differences are unlikely to have a substantial impact on results.
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	The statistical methods were clearly described by the study authors and were appropriate for datasets.
	Metric 24: Reporting of Data	Low	× 2	6	Some data are reported incompletely. For example, incidences for reported clinical signs were not reported. Severity scores were not reported for non-neoplastic data.
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 23: **Animal toxicity evaluation results for Wang et al 2017 for a 24-week study on autoimmune outcomes**

Study Citation:	Wang, G., Wang, J., Ansari, G. A. S., Khan, M. F. (2017). Autoimmune potential of perchloroethylene: Role of lipid-derived aldehydes Toxicology and Applied Pharmacology, 333 76-83
Data Type:	Autoimmunity for perc
HERO ID:	4724508

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 (perchloroethylene or tetrachloroethylene).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (Sigma-Aldrich). A batch number was not reported; however, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported (>99%).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	An appropriate negative control group was used. Control animals were administered drinking water containing 1% Alkamuls EL-620 emulsifier only.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric was not applicable to the study type.
	Metric 6:	Randomized Allocation	Low	× 1	3	The study did not report how animals were allocated to study groups. The study indicates only that "mice were divided into 6 groups of 6 each."
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The study indicates that perc was dissolved in drinking water containing 1% Alkamuls EL-620 emulsifier, and that water was changed on alternate days. Additional details regarding the storage of perc were not expected to significantly impact the study results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Animals were exposed consistently across study groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Deficiencies in the reporting of administered doses occurred (i.e., no information on animal body weight or intake were provided). The study indicates that the consumption of perc-containing drinking water was measured and that mice were weighed weekly to monitor body weight changes; however, these data were not provided in the report.

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Study Citation:	Wang, G., Wang, J., Ansari, G. A. S., Khan, M. F. (2017). Autoimmune potential of perchloroethylene: Role of lipid-derived aldehydes Toxicology and Applied Pharmacology, 333 76-83
Data Type:	Autoimmunity for perc
HERO ID:	4724508

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure duration (i.e., 12, 18, and 24 weeks) was clearly specified and were reported to be appropriate for the outcome of interest (i.e., administered for a time period prior to the development of autoimmune disease).
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Only one concentration was tested (0.5 mg/mL). A rationale for this dose was provided (i.e., the dose selected was occupationally relevant based on the 8-hour PEL established by OSHA). The dose selected permitted the evaluation of effects over the time course of the experiment (12, 18, and 24 weeks).
	Metric 12:	Exposure Route and Method	High	× 1	1	The route/method of exposure was reported (perc in drinking water) and is appropriate for the test substance. The study indicated perc is a frequent contaminant in drinking water samples.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The strain and sex of mice utilized in the study (female MRL+/+ mice) were selected owing to higher susceptibility and prevalance of autoimmune diseases. The mouse mouse strain used is a model of systemic lupus erythematosus (SLE). Information pertaining to the species, strain, sex, age (5 weeks), and source (Jackson Laboratories) were reported; however, information pertaining to health status and starting body weights were not specified.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported (e.g., temperature, humidity, light- dark cycle) and were adequate and the same for control and exposed populations, such that the only difference was exposure.
	Metric 15:	Number per Group	Medium	× 1	2	The reported number of animals per study group was lower than the typical number used in studies of the same or similar type (e.g., 6/group), but sufficient for statistical analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported (e.g., quantification of auto-antibodies in the serum) and was sensitive for the outcome of interest (autoimmune response).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.

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Study Citation:	Wang, G., Wang, J., Ansari, G. A. S., Khan, M. F. (2017). Autoimmune potential of perchloroethylene: Role of lipid-derived aldehydes Toxicology and Applied Pharmacology, 333 76-83
Data Type:	Autoimmunity for perc
HERO ID:	4724508

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 18:	Sampling Adequacy	High	× 1	1	Details regarding sampling for the outcomes of interest were reported and appropriate. Endpoints were presumably evaluated in all animals/group (although n was not explicitly specified in the figure legends).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study type. Subjective outcomes were not assessed in the study.
	Metric 20:	Negative Control Response	Medium	× 1	2	The biological responses of the negative control group were adequate. An autoimmune response was observed in the negative control group; however, the response was such that effects due to the test substance could be reasonably observed (i.e., the test substance significantly exacerbated the autoimmune response),
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in test design and procedures were reported. Initial body weights and intake were not specified in the study report.
	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 datasets.
	Metric 24:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes 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.

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

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

6 Genetic toxicity studies

Table 24: **Animal toxicity evaluation results for Schumann et al 1980 for rat and mouse oral and inhalation exposure study on DNA alkylation**

Study Citation:	A. M. Schumann, J. F. Quast, P. G. Watanabe (1980). The pharmacokinetics and macromolecular interactions of perchloroethylene in mice and rats as related to oncogenicity Toxicology and Applied Pharmacology, 55(2,2), 207-219
Data Type:	DNA alkylation in rat and mouse liver (oral and inhalation)
HERO ID:	58169

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.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified as Dow Chemical Company.
	Metric 3:	Test Substance Purity	High	× 1	1	>99% as determined by GC.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Negative controls are not needed for DNA alkylation with radiolabeled Perc.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	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	The method and equipment used to generate the test substance as a vapor were reported and appropriate. Preparation in corn oil was described for oral gavage. Storage was not indicated; however, only a single dose was used.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Analytical inhalation concentrations were reported and were within 4% of the the target concentrations (measured by GC). Oral doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	A single 6-h exposure or gavage dose is adequate.

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Study Citation: A. M. Schumann, J. F. Quast, P. G. Watanabe (1980). The pharmacokinetics and macromolecular interactions of perchloroethylene in mice and rats as related to oncogenicity Toxicology and Applied Pharmacology, 55(2,2), 207-219
Data Type: DNA alkylation in rat and mouse liver (oral and inhalation)
HERO ID: 58169

Domain		Metric	Rating†	MWF*	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	Unacceptable	× 1	4	A single 600 ppm concentration or 500 mg/kg dose was used. This is considered to have substantially impacted results, as negative responses were observed (below the limit of detection) and it was not apparent that adverse health outcomes were observed, indicating toxicity. In another experiment in this paper, oral gavage of 1000 mg/kg/day for 11 days did not affect body weights in mice or rats. Therefore, it is not clear that the doses chosen were high enough to assess this endpoint.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Whole body chamber was used; Perc may condense. Oral gavage was considered appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and starting body weight were reported, and the test animals was obtained from a commercial source. Age and health status were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported and appropriate.
	Metric 15:	Number per Group	Medium	× 1	2	n=3
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported and was sensitive for DNA alkylation (covalent binding of radiolabeled Perc).
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Only one study group was used (600 ppm or 500 mg/kg).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 20:	Negative Control Response	Not Rated	NA	NA	Negative controls were not used.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Not Rated	NA	NA	Only one study group was used (600 ppm or 500 mg/kg).
	Metric 22:	Health Outcomes Unrelated to Exposure	Not Rated	NA	NA	DNA alkylation in rat and mouse liver (oral and inhalation).
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	Not Rated	NA	NA	DNA alkylation in rat and mouse liver (oral and inhalation).

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Study Citation:	A. M. Schumann, J. F. Quast, P. G. Watanabe (1980). The pharmacokinetics and macromolecular interactions of perchloroethylene in mice and rats as related to oncogenicity Toxicology and Applied Pharmacology, 55(2,2), 207-219
Data Type:	DNA alkylation in rat and mouse liver (oral and inhalation)
HERO ID:	58169

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Reporting of Data	Not Rated	NA	NA	No binding to DNA was detected (below limit of detection; no quantitative data).
Overall Quality Determination‡		Unacceptable⋆⋆		1.5	
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 25: **Animal toxicity evaluation results for Millman et al 1988 for acute oral study in rats on liver outcomes**

Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	Perc GGT+ foci initiation and promotion protocols
HERO ID:	200479

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by chemical name.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer was specified.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was reported as a range for multiple compounds (97-99% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Vehicle controls were used (corn oil).
	Metric 5:	Positive Controls	High	× 1	1	Diethylnitrosamine initiation followed by phenobarbital promotion was utilized as a positive control and was appropriate for the outcome of interest. Positive controls yielded positive responses.
	Metric 6:	Randomized Allocation	High	× 1	1	Randomization was indicated.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation in corn oil was indicated, but storage was not described.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Gavage volume was indicated and appropriate.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	MTD 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 the initiation/promotion study types.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	A single dose was used (specified as the MTD).
	Metric 12:	Exposure Route and Method	High	× 1	1	Oral gavage in corn oil is appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source of the test animal, age and health status were not reported.
	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	9-10 rats/group
Domain 5: Outcome Assessment						

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Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	Perc GGT+ foci initiation and promotion protocols
HERO ID:	200479

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	Due to incomplete reporting, it was unclear whether methods were sensitive for the outcome of interest. Staining procedures were not described (cited to another publication).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Animals were sacrificed at a consistent timepoint.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Livers were examined for all exposed animals. It appears that only one slide per liver was assessed. The standard deviation values in Tables 3 and 4 represent variation across square centimeters of the tissue.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not required for initial histopathology evaluation.
	Metric 20:	Negative Control Response	High	× 1	1	Negative controls responded appropriately.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight and food/water consumption were not reported for each study group.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	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 analysis was not described. However, sufficient summary data is provided, enabling independent statistical analysis.
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for each exposure group.
Overall Quality Determination‡			High		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 26: **Animal toxicity evaluation results for Cederberg et al 2010 for mouse study on DNA damage in liver and kidney**

Study Citation:	H. Cederberg, J. Henriksson, M. L. Binderup (2010). DNA damage detected by the alkaline comet assay in the liver of mice after oral administration of tetrachloroethylene Mutagenesis, 25(2,2), 133-138
Data Type:	DNA damage mouse liver and kidney
HERO ID:	628833

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 CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	99.96% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Vehicle controls were used.
	Metric 5:	Positive Controls	High	× 1	1	EMS was used as a positive control and responded appropriately.
	Metric 6:	Randomized Allocation	High	× 1	1	The study reported randomized allocation of animals to treatment groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Dosing solutions were prepared in corn oil prior to dosing. Storage of test substance between dose administrations was not reported, but this is not expected to have had a substantial impact on results.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Gavage volume was not excessive. Exposures were administered consistently across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	2 doses, 24h apart was adequate for the outcome of interest.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 dose groups; levels were justified based on body weight loss in previous studies.
	Metric 12:	Exposure Route and Method	High	× 1	1	Oral gavage in corn oil is appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain, sex, age and commercial source were reported. Body weight and health status were not described.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were well-reported and appropriate.
	Metric 15:	Number per Group	High	× 1	1	6/group
Domain 5: Outcome Assessment						

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Study Citation:	H. Cederberg, J. Henriksson, M. L. Binderup (2010). DNA damage detected by the alkaline comet assay in the liver of mice after oral administration of tetrachloroethylene Mutagenesis, 25(2,2), 133-138
Data Type:	DNA damage mouse liver and kidney
HERO ID:	628833

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported and senstive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Medium	× 1	2	Scoring was assessed for 100 cells per animal per tissue (50 cells on each of two slides). This is considered somewhat lacking in comparison to current standards and guidelines (150 cells/animal is recommended).
	Metric 19:	Blinding of Assessors	High	× 1	1	Blinding was reported.
	Metric 20:	Negative Control Response	High	× 1	1	The negative control responded appropriately.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weight, food and water consumption were not reported for all groups. Given the short term duration of the study, this is not expected to have substantially impacted results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	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 reported and appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Group means and individual animal data were presented.
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 27: **Animal toxicity evaluation results for Mazzullo et al 1987 for DNA and protein binding study**

Study Citation:	M. Mazzullo, S. Grilli, G. Lattanzi, G. Prodi, M. P. Turina, A. Colacci (1987). Evidence of DNA binding activity of perchloroethylene Research Communications in Chemical Pathology and Pharmacology, 58(2,2), 215-235
Data Type:	In vivo/ex vivo DNA, RNA, and protein binding for Perc
HERO ID:	628902

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as 14C-Perchloroethylene (abbreviated [U-14C]-PCE).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Radiochemical purity of Perc was reported to 97%. PCE impurity was due to "hexachloroethane utilized in its synthesis". It was unclear whether any hexachloroethane was radiolabeled. Hexachloroethane has been previously linked to DNA binding (Lattanzi et al. 1987).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	Negative control animals were not included in the study design. However, negative control animals are not necessarily required for these binding assays.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 6:	Randomized Allocation	Low	× 1	3	The method of animal allocation was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Low	× 1	3	It is not clear whether the test substance was diluted in a vehicle for i.p. administration or if it was injected neat.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Although there was only one dose level and no negative or positive control animals, the test substance was administered consistently across species (mice and rats).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The dose was reported without ambiguity in terms of absolute dose (8.70 umol/kg) and radioactivity (127 uCi/kg).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Samples were collected 22 hours after injection of the test substance, which is appropriate for the study design.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	There was a single dose level in this study. The dose appeared to be adequate to assess the outcome of interest.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure was reported and appropriate.

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Study Citation:	M. Mazzullo, S. Grilli, G. Lattanzi, G. Prodi, M. P. Turina, A. Colacci (1987). Evidence of DNA binding activity of perchloroethylene Research Communications in Chemical Pathology and Pharmacology, 58(2,2), 215-235
Data Type:	In vivo/ex vivo DNA, RNA, and protein binding for Perc
HERO ID:	628902

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 4: Test Organism					
Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species, strain, sex, life stage (adult; age not specified), and starting body weight ranges were reported. Test animal health status was not reported, but this is not expected to have substantially impacted results.
Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry conditions were not reported.
Metric 15:	Number per Group	High	× 1	1	The number of animals per group was reported and appropriate (6 rats, 16 mice).
Domain 5: Outcome Assessment					
Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Pooling different numbers of livers for rats (n = 6) versus mice (n = 16) may have affected results.
Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design.
Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
Metric 20:	Negative Control Response	Not Rated	NA	NA	Negative controls were not used.
Domain 6: Confounding / Variable Control					
Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food and 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	Low	× 1	3	No statistical analysis was performed; however, independent statistical analysis may be performed for liver endpoints with the summary data provided (mean +/- SEM). Variance data were not provided for kidney, lung or stomach DNA, RNA, or protein binding.
Metric 24:	Reporting of Data	Medium	× 2	4	It was unclear how the reported means and SEMs reflect pooling of organs prior to analysis (Table 1).
Overall Quality Determination‡		Medium		1.8	
Extracted		Yes			

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Study Citation:	M. Mazzullo, S. Grilli, G. Lattanzi, G. Prodi, M. P. Turina, A. Colacci (1987). Evidence of DNA binding activity of perchloroethylene Research Communications in Chemical Pathology and Pharmacology, 58(2,2), 215-235
Data Type:	In vivo/ex vivo DNA, RNA, and protein binding for Perc
HERO ID:	628902

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 28: **Animal toxicity evaluation results for Murakami and Horikawa 1995 for mouse micronuclei study**

Study Citation:	K. Murakami, K. Horikawa (1995). The induction of micronuclei in mice hepatocytes and reticulocytes by tetrachloroethylene Chemosphere, 31(7,7), 3733-3739
Data Type:	In vivo micronuclei for Perc
HERO ID:	628931

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as tetrachloroethylene (abbreviated "tetra").
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was identified.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported to be 99.8%. It was also reported that the test substance was checked for specific impurities (epichlorohydrin, chloroform, and carbon tetrachloride) by gas chromatography and was not found to have these impurities.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were treated with vehicle (olive oil) by the same route (i.p. injection).
	Metric 5:	Positive Controls	High	× 1	1	Mitomycin C (for assessing micronucleated reticulocytes) and diethylnitrosamine (for assessing micronucleated hepatocytes) were used as positive controls and yielded positive responses.
	Metric 6:	Randomized Allocation	Low	× 1	3	The method of animal allocation was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	The preparation of the test substance (dissolved in olive oil) was reported. The test substance storage was not reported, but this is appropriate given the study design (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	The administration of test substance was consistent among treatment groups (equivalent amount of olive oil vehicle).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	For assessment of micronucleated reticulocytes, blood samples were collected at 0, 24, 48, and 72 hours after test substance administration. For assessment of micronucleated hepatocytes, partial hepatectomy was conducted, with test substance administration occurring 24 hours later. Hepatocytes were isolated 72 hours after injection. The timeline is appropriate for the endpoints of interest.

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Study Citation: K. Murakami, K. Horikawa (1995). The induction of micronuclei in mice hepatocytes and reticulocytes by tetrachloroethylene Chemosphere, 31(7,7), 3733-3739
Data Type: In vivo micronuclei for Perc
HERO ID: 628931

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose spacing were appropriate. The doses are considered to be adequate for these endpoints, as a positive response was observed at the mid and high dose in isolated hepatocytes. Doses were justified by the results of a range finding study.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were reported and appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species, strain, sex, and age were reported. The test animal health status and starting body weight ranges were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Medium	× 1	2	The temperature and light-dark cycle were reported. The humidity of the animal rooms was not reported. This is not considered to have substantially impacted results.
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group (n = 5) was reported and appropriate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	Low	× 1	3	The sampling (1,000 reticulocytes and 1,000 hepatocytes analyzed for micronuclei per animal) is not considered adequate; current standards call for 4,000 reticulocytes per animal.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 20:	Negative Control Response	High	× 1	1	Both vehicle controls and 0-hour controls yielded negative responses.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food and 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						

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Study Citation:	K. Murakami, K. Horikawa (1995). The induction of micronuclei in mice hepatocytes and reticulocytes by tetrachloroethylene Chemosphere, 31(7,7), 3733-3739
Data Type:	In vivo micronuclei for Perc
HERO ID:	628931

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 23: Statistical Methods	High	× 1	1	It is unclear whether Kastenbaum & Bowman's test is appropriate for all data in this study (e.g. the reticulocyte data with multiple timepoints). However, summary data (mean, standard deviation, and sample size) is provided and enables independent statistical analysis.
	Metric 24: Reporting of Data	High	× 2	2	All data were reported adequately.
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: **Animal toxicity evaluation results for Toraason et al 1999 for acute study in rats on DNA damage**

Study Citation:	M. Toraason, J. Clark, D. Dankovic, P. Mathias, S. Skaggs, C. Walker, D. Werren (1999). Oxidative stress and DNA damage in Fischer rats following acute exposure to trichloroethylene or perchloroethylene Toxicology, 138(1,1), 43-53					
Data Type:	DNA damage for Perc (8OHdG adducts)					
HERO ID:	628948					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as perchloroethylene (PERC).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be 99.5% pure (spectrophotometric grade).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent negative controls were treated with a 1:4 v/v ratio of Alkamuls® to water.
	Metric 5:	Positive Controls	High	× 1	1	Concurrent positive controls were treated with 2-nitropropane in vehicle. Positive controls responded appropriately.
	Metric 6:	Randomized Allocation	High	× 1	1	It was reported that animals were randomly allocated into the treatment groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was reported. Test substance storage was not reported, but this is appropriate given the study design (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure parameters were consistent among treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency (single-dose administration) and duration (12 hr and 24 hr urine sample collection; 24 hr sacrifice) were reported and appropriate.

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Study Citation:	M. Toraason, J. Clark, D. Dankovic, P. Mathias, S. Skaggs, C. Walker, D. Werren (1999). Oxidative stress and DNA damage in Fischer rats following acute exposure to trichloroethylene or perchloroethylene Toxicology, 138(1,1), 43-53
Data Type:	DNA damage for Perc (8OHdG adducts)
HERO ID:	628948

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	The number of exposure groups and dose spacing was reported and appropriate. It should be noted that only the mid-dose (500 mg/kg) was tested for liver and lymphocyte 8OHdG due to cost restraints. However, this dose to be tested for these endpoints was selected based on the highest TBARS values (oxidative stress). Furthermore, although negative results were observed after 500 mg/kg Perc, this dose was considered to be sufficient for this endpoint due to the health effects noted at this dose (loss of righting reflex, reduced body weight and reduced relative liver weight at 24 hr post-injection).
	Metric 12:	Exposure Route and Method	High	× 1	1	The exposure route was reported and appropriate for the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Test animal species, strain, sex, and starting body weight range were reported. Test animal health status and age were not reported, but this is not expected to have substantially impacted results.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	It was reported that rats were housed individually, but no details regarding temperature, humidity, or light-dark cycles were reported.
	Metric 15:	Number per Group	High	× 1	1	Each treatment group consisted of n = 6 rats.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was appropriate for the endpoint of interest (DNA damage in liver and lymphocytes). The detection of 8OHdG in urine via HPLC-EC was considered exploratory and was not assessed for this review.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome was assessed consistently across treatment groups.
	Metric 18:	Sampling Adequacy	Low	× 1	3	It was unclear how many technical replicates per animal were included in the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 20:	Negative Control Response	High	× 1	1	The negative controls responded appropriately.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial body weight, food and water intake were not reported for each group.

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Study Citation:	M. Toraason, J. Clark, D. Dankovic, P. Mathias, S. Skaggs, C. Walker, D. Werren (1999). Oxidative stress and DNA damage in Fischer rats following acute exposure to trichloroethylene or perchloroethylene Toxicology, 138(1,1), 43-53
Data Type:	DNA damage for Perc (8OHdG adducts)
HERO ID:	628948

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	Medium	× 1	2	Data were appropriately analyzed by ANOVA; however, it was not specified whether a one-way or two-way ANOVA was used, and the post-hoc test was not specified.
	Metric 24:	Reporting of Data	Medium	× 2	4	Only one of three dose levels were tested for the liver and lymphocyte 8OHdG endpoint.
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 30: **Animal toxicity evaluation results for Valencia et al 1985 for drosophila sex-linked recessive lethal test study**

Study Citation:	R. Valencia, J. M. Mason, R. C. Woodruff, S. Zimmering (1985). Chemical mutagenesis testing in Drosophila. III. Results of 48 coded compounds tested for the National Toxicology Program Environmental Mutagenesis, 7(3,3), 325-348
Data Type:	Sex-linked recessive lethal test in Drosophila for TCE
HERO ID:	629907

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 (tetrachloroethylene). A CASRN and structure was also provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported (batch or lot number also presumably included).
	Metric 3:	Test Substance Purity	Medium	× 1	2	The grade (technical) but not purity of the test substance was reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study authors reported using concurrent negative controls; however, it was not clear if negative controls were untreated or solvent-only controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not reported; however, positive results were observed for substances tested in the study (showing that the assay is capable of detecting a response). In addition, it was indicated that the first paper in this series (Woodruff et al. 1984) showed results for two positive controls (to indicate that data from three laboratories were compatible).
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	This metric is not applicable to study designs utilizing Drosophila.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The test substance preparation was reported adequately (dissolved in 10% EtOH for both feeding and injection exposures). Test substance storage was not reported (but is not expected to impact the study results).
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Exposures appeared to be administered consistently across treatment groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (Table 2).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure duration prior to mating was reported and appeared to be appropriate for the study design.

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Study Citation:	R. Valencia, J. M. Mason, R. C. Woodruff, S. Zimmering (1985). Chemical mutagenesis testing in Drosophila. III. Results of 48 coded compounds tested for the National Toxicology Program Environmental Mutagenesis, 7(3,3), 325-348
Data Type:	Sex-linked recessive lethal test in Drosophila for TCE
HERO ID:	629907

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	There was only one dose administered for each route (feeding and injection), but detailed preliminary studies were described and doses were chosen based on limiting factors including solubility, toxicity, ingestion, and male sterility. Therefore the doses chosen were considered appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	Feeding (then injection, if results were negative) were considered appropriate routes to evaluate the outcome of interest.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The Drosophila stocks and genetic crosses were described in more detail in cited references, but are routinely used for the outcome of interest and appeared appropriate.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Animal husbandry was not reported.
	Metric 15:	Number per Group	Medium	× 1	2	The study indicated that males were mated individually to 3 harems of females to produce 3 broods. To reduce the chances of recovering several lethals from the same male, no more than 40 F1 females were mated individually from each brood of each male.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was sensitive and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	It appears that the outcome was assessed consistently across treated and control groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	High	× 1	1	The study indicated that chemicals were coded and were identified only after test results were reported.
	Metric 20:	Negative Control Response	High	× 1	1	The negative control response appeared appropriate (low numbers of lethals from control broods).
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding variables in initial study parameters were reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	No confounding variables unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						

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Study Citation:	R. Valencia, J. M. Mason, R. C. Woodruff, S. Zimmering (1985). Chemical mutagenesis testing in Drosophila. III. Results of 48 coded compounds tested for the National Toxicology Program Environmental Mutagenesis, 7(3,3), 325-348
Data Type:	Sex-linked recessive lethal test in Drosophila for TCE
HERO ID:	629907

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Statistical Methods	High	× 1	1	The statistical methods used were appropriate.
	Metric 24:	Reporting of Data	High	× 2	2	Data was reported for all treatment groups and endpoints.
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: **Animal toxicity evaluation results for Walles 1986 for SSB in DNA study**

Study Citation:	S. A. S. Walles (1986). Induction of single-strand breaks in dna of mice by trichloroethylene and tetrachloroethylene Toxicology Letters, 31(1,1), 31-35
Data Type:	SSB in DNA 1 hr and 24 hr - Perc
HERO ID:	629915

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as tetrachloroethylene (PER).
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer of test substance was identified.
	Metric 3:	Test Substance Purity	High	× 1	1	Perc was reported to be 99.8% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Concurrent solvent control group was included.
	Metric 5:	Positive Controls	Medium	× 1	2	The study provided data for MMS and <U+0264>-radiation as demonstration of the method used (i.e., alkaline unwinding); however, these data were not obtained concurrently.
	Metric 6:	Randomized Allocation	Low	× 1	3	Random allocation of mice was not reported.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation of test substance is reported. Test substance storage is not reported, but this is appropriate given the study design (single-dose administration).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across exposure groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses are reported without ambiguity in Figures 1 and 2.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were reported and appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	Dose response was obtained in liver and kidneys; however, it is not clear if lowest dose was low enough. Similarly, in the absence of overt toxicity of adverse health effects, it is not clear if the response may have been obtained in negative tissue (lungs) at a higher dose.
	Metric 12:	Exposure Route and Method	High	× 1	1	Exposure route and method were appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source of the test animal was not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.

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Study Citation:	S. A. S. Walles (1986). Induction of single-strand breaks in dna of mice by trichloroethylene and tetrachloroethylene Toxicology Letters, 31(1,1), 31-35
Data Type:	SSB in DNA 1 hr and 24 hr - Perc
HERO ID:	629915

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of animals per group can be found in Figures 1 and 2 and was appropriate for both test substances.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Methods are cited to other studies (Walles and Erixon, 1984; Ahnstrom and Erixon, 1973).
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Methods are cited to other studies (Walles and Erixon, 1984; Ahnstrom and Erixon, 1973).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Methods are cited to other studies (Walles and Erixon, 1984; Ahnstrom and Erixon, 1973).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not necessary for this study.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control response was adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no confounding variables reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Data on health outcomes were not reported. Given the study length (1hr and 24 hrs) and the nature of study, it is unlikely to have had a substantial impacted on the results.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analyses were performed with Student's t-test. This is considered to be appropriate. Independent data analysis could not be conducted due to uncertainty about number of animals per group (a range is given).
	Metric 24:	Reporting of Data	Medium	× 2	4	Data regarding 24hr timepoint was reported for only one of the four doses administered.
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 =≥ 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 32: **Animal toxicity evaluation results for Schumann et al 1980 for rat and mouse oral exposure study on DNA synthesis**

Study Citation:	A. M. Schumann, J. F. Quast, P. G. Watanabe (1980). The pharmacokinetics and macromolecular interactions of perchloroethylene in mice and rats as related to oncogenicity Toxicology and Applied Pharmacology, 55(2,2), 207-219
Data Type:	DNA synthesis in rat and mouse liver (oral)
HERO ID:	58169

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.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified as Dow Chemical Company.
	Metric 3:	Test Substance Purity	High	× 1	1	>99% as determined by GC.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Vehicle controls were used.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	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	Low	× 1	3	Preparation in corn oil was described for oral gavage. Storage conditions were not indicated.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Oral doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	12 doses in 16 days or 11 doses in 11 days.
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	For the 11 day exposure , 4 treatment groups were used.
	Metric 12:	Exposure Route and Method	High	× 1	1	Oral gavage was reported and suited to the test substance.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	The test animal species, strain, sex, and starting body weight were reported, and the test animals was obtained from a commercial source. Age and health status were not reported.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were reported and appropriate.
	Metric 15:	Number per Group	Medium	× 1	2	n=3-7/group
Domain 5: Outcome Assessment						

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Study Citation:	A. M. Schumann, J. F. Quast, P. G. Watanabe (1980). The pharmacokinetics and macromolecular interactions of perchloroethylene in mice and rats as related to oncogenicity Toxicology and Applied Pharmacology, 55(2,2), 207-219
Data Type:	DNA synthesis in rat and mouse liver (oral)
HERO ID:	58169

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported and was sensitive for DNA synthesis (3H-thymidine incorporation).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently aross groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control response seemed appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial body weight and food/water intake were not reported ; however, this is not expected to affect results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Attrition and/or health outcomes unrelated to exposure for each study group were not reported; however, this is unlikely to have a substantial impact on results.
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 for repeat dose oral exposure 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 33: **Animal toxicity evaluation results for Potter et al 1996 for DNA synthesis study**

Study Citation:	C. L. Potter, L. W. Chang, A. B. Deangelo, F. B. Daniel (1996). Effects of four trihalomethanes on DNA strand breaks, renal hyaline droplet formation and serum testosterone in male F-344 rats Cancer Letters, 106(2,2), 235-242
Data Type:	DNA synthesis
HERO ID:	630895

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as perchloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	Source of test substance was Aldrich Chemical Co, Inc (Milwaukee, WI).
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity or grade of test substance is not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The negative control group received vehicle alone.
	Metric 5:	Positive Controls	Not Rated	NA	NA	The results from diethylnitrosamine and dimethylnitrosamine-treated animals were not reported for the DNA synthesis endpoint (it was inferred that these treatment groups were analyzed for this endpoint based on information provided in Section 2.3, Histology and autoradiography). However, a positive control is not required for this assay (radioactive tritiated thymidine as detection system).
	Metric 6:	Randomized Allocation	High	× 1	1	Rats were randomly allocated into treatment groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation of test substance was reported and analyzed by gas chromatography for decrement throughout study.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Test substance was consistently administered.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose was reported without ambiguity (1000 mg/kg)
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration (3 or 7 days) were reported and appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Dose used was based on previous work in bioassays, however no response was seen at this dose. It is unclear if a higher dose would elicit a response.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Age and health status of animals was not reported. Animals were purchased from Charles River Laboratories, Inc (Portage, MI).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were sufficiently reported.

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Study Citation:	C. L. Potter, L. W. Chang, A. B. Deangelo, F. B. Daniel (1996). Effects of four trihalomethanes on DNA strand breaks, renal hyaline droplet formation and serum testosterone in male F-344 rats Cancer Letters, 106(2,2), 235-242
Data Type:	DNA synthesis
HERO ID:	630895

Domain	Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 15: Number per Group	High	× 1	1	Four rats were studied per treatment group.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology is partially described and cited elsewhere, but appeared appropriate for the outcome of interest.
	Metric 17: Consistency of Outcome Assessment	Medium	× 1	2	There was incomplete reporting of minor details of outcome assessment protocol. This is unlikely to have a substantial impact on results.
	Metric 18: Sampling Adequacy	Low	× 1	3	It is unclear how many technical replicates (i.e. cells per slide or slides per animal) were included in the study design.
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	Blinding was not applicable for this study.
	Metric 20: Negative Control Response	High	× 1	1	Negative control response was appropriate.
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables in test design and procedures were identified.
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	× 1	2	Health outcomes unrelated to exposure were not reported or identified.
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Statistical analysis was described, but it is not clear that Levene's test for multiple comparisons is acceptable. However, mean and standard deviation could be estimated from the graph, enabling independent analysis.
	Metric 24: Reporting of Data	High	× 2	2	Outcome data were all presented.
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 34: **Animal toxicity evaluation results for Potter et al 1996 for DNA strand break study**

Study Citation:	C. L. Potter, L. W. Chang, A. B. Deangelo, F. B. Daniel (1996). Effects of four trihalomethanes on DNA strand breaks, renal hyaline droplet formation and serum testosterone in male F-344 rats Cancer Letters, 106(2,2), 235-242
Data Type:	DNA strand break
HERO ID:	630895

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as perchloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	Source of test substance was Aldrich Chemical Co, Inc (Milwaukee, WI).
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity or grade of test substance is not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The negative control group received vehicle alone.
	Metric 5:	Positive Controls	High	× 1	1	Diethylnitrosamine and dimethylnitrosamine were utilized as positive controls and yielded positive responses.
	Metric 6:	Randomized Allocation	High	× 1	1	Rats were randomly allocated into treatment groups.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation of test substance was reported and analyzed by gas chromatography for decrement throughout study.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Test substance was consistently administered.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Dose was reported without ambiguity (1000 mg/kg)
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration (1 day) were reported and appropriate.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Dose used was based on previous work in bioassays, however no response was seen at this dose. It is unclear if a higher dose would elicit a response.
	Metric 12:	Exposure Route and Method	High	× 1	1	The route and method of exposure were appropriate.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Age and health status of animals was not reported. Animals were purchased from Charles River Laboratories, Inc (Portage, MI).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were sufficiently reported.
	Metric 15:	Number per Group	High	× 1	1	Four rats were studied per treatment group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology is partially described and cited elsewhere, but appeared appropriate for the outcome of interest.

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Study Citation:	C. L. Potter, L. W. Chang, A. B. Deangelo, F. B. Daniel (1996). Effects of four trihalomethanes on DNA strand breaks, renal hyaline droplet formation and serum testosterone in male F-344 rats Cancer Letters, 106(2,2), 235-242
Data Type:	DNA strand break
HERO ID:	630895

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There was incomplete reporting of minor details of outcome assessment protocol. This is unlikely to have a substantial impact on results.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not applicable for this study.
	Metric 20:	Negative Control Response	High	× 1	1	Negative control response was appropriate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables in test design and procedures were identified.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	Health outcomes unrelated to exposure were not reported or identified.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was described, but it is not clear that Levene's test for multiple comparisons is acceptable. However, sufficient summary data are provided, enabling independent analysis.
	Metric 24:	Reporting of Data	High	× 2	2	Outcome data were all presented.
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 35: **Animal toxicity evaluation results for NTP 1986 for drosophila sex-linked recessive lethal test study**

Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	SLRL mutations in Drosophila
HERO ID:	632655

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Assumed to be the same as the rat and mouse studies (high-purity tetrachloroethylene, Dowper stabilized)
	Metric 2:	Test Substance Source	High	× 1	1	Assumed to be the same as the rat and mouse studies (Dow Chemical, lot TA03116F-01; purity and identity analyses conducted)
	Metric 3:	Test Substance Purity	High	× 1	1	Assumed to be the same as the rat and mouse studies (confirmed analytically - approximately 99.9%)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Negative controls were used, but it is not clear whether they were vehicle or untreated controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not needed for study type.
	Metric 6:	Randomized Allocation	Not Rated	NA	NA	Not needed for study type.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	Perc was found to be stable for 2 weeks at 60" C (Appendix H). Perc was prepared in 5% sucrose or 0.7% NaCl. Not clear if sucrose was replaced daily.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was assumed to be consistent across groups.
	Metric 9:	Reporting of Doses/Concentrations	Medium	× 2	4	Dose was reported as ppm (assumed to be concentration in sucrose or NaCl).
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were reported and appropriate for the study type.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single dose groups plus controls
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Feeding and injection were appropriate routes; however, it is not clear whether sucrose was replaced daily to account for volatilization.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source of the test animal was not reported (species, strain, substrain, and age were reported).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.

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Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	SLRL mutations in Drosophila
HERO ID:	632655

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	Not Rated	NA	NA	The initial number of animals per group was not reported, but the study methods were cited to another publication (Abrahamson and Lewis, 1971).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Study methods were cited to another publication (Abrahamson and Lewis, 1971).
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Study methods were cited to another publication (Abrahamson and Lewis, 1971).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Study methods were cited to another publication (Abrahamson and Lewis, 1971).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Evaluated endpoints did not require blinding
	Metric 20:	Negative Control Response	High	× 1	1	Control responses reported and appeared to be acceptable
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	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	Medium	× 1	2	Statistics were performed, but methods were cited to another publication (Margolin et al. 1983).
	Metric 24:	Reporting of Data	High	× 2	2	Data were reported for all groups and matings.
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 $=\geq 1$ 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 for Galloway et al 1987 for Chinese hamster ovary cell sister chromatid exchange study**

Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	Perc in vitro SCE
HERO ID:	7768

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified using established nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The test substances were obtained from Litton Bionetics, Inc.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substances were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Solvent controls were employed appropriately.
	Metric 5:	Positive Controls	High	× 2	2	Two positive controls were employed (triethylenemelamine or mitomycin C and cyclophosphamide); their response was appropriate (significant increase in chromosomal aberrations).
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were well described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to this study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	General information regarding test substance preparation was included (e.g., dissolving in solvent immediately before use), but storage conditions were not provided.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Information regarding exposure administration was reported and consistency of administration across groups is inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure doses were reported for each trial.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was clearly stated and appropriate for the endpoint.
	Metric 12:	Exposure Route and Method	High	× 1	1	Dose selection was described in detail and based on preliminary growth inhibition tests, followed by observations of cell monolayer confluence and mitotic activity to maximize available metaphase cells. The number of exposure groups was consistent for the test.
	Metric 13:	Metabolic Activation	High	× 1	1	Tests were run with and without metabolic activation. Preparation of S9 mix was described in detail.
Domain 4: Test Model						

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Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	Perc in vitro SCE
HERO ID:	7768

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 14:	Test Model	High	× 2	2	Test models were described in detail and appropriate for the endpoints assessed.
	Metric 15:	Number per Group	Low	× 1	3	There was only one study group for each of the three exposure concentrations tests (i.e., no replicates).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology addressed the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment protocol was consistent across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The number of cells/dose was reported and is appropriate (50 cells/dose).
	Metric 19:	Blinding of Assessors	High	× 1	1	Test substance was supplied under code; assessors did not know its identity until after scoring.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no confounding variables in test design or procedures that were reported by study authors.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	There were no confounding variables reported unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analyses were clearly described and presented in results tables.
	Metric 23:	Data Interpretation	High	× 2	2	Data were reported in such a way as to allow interpretation of test results.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints such as induction of cell death and delay in cell cycle progression were noted, and selected exposure doses were based on relation to toxicity. However, methods of measurement for specific cytotoxicity endpoints were not described.
	Metric 25:	Reporting of Data	High	× 2	2	Data were presented for percent cells with aberrations in three ways for each exposure concentration: total, simple, and complex aberrations.
Overall Quality Determination[‡]			High		1.3	
Extracted			Yes			

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Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	Perc in vitro SCE
HERO ID:	7768

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 37: **In vitro evaluation results for Galloway et al 1987 for Chinese hamster ovary cell chromosomal aberration study**

Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	Perc in vitro chromosomal aberration
HERO ID:	7768

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substances were identified using established nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The test substances were obtained from Litton Bionetics, Inc.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of the test substances were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Solvent controls were employed appropriately.
	Metric 5:	Positive Controls	High	× 2	2	Two positive controls were employed (triethylenemelamine or mitomycin C and cyclophosphamide); their response was appropriate (significant increase in chromosomal aberrations).
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were well described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to this study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	General information regarding test substance preparation was included (e.g., dissolving in solvent immediately before use), but storage conditions were not provided.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Information regarding exposure administration was reported and consistency of administration across groups is inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure doses were reported for each trial.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was clearly stated and appropriate for the endpoint.
	Metric 12:	Exposure Route and Method	High	× 1	1	Dose selection was described in detail and based on preliminary growth inhibition tests, followed by observations of cell monolayer confluence and mitotic activity to maximize available metaphase cells. The number of exposure groups was consistent for the test.
	Metric 13:	Metabolic Activation	High	× 1	1	Tests were run with and without metabolic activation. Preparation of S9 mix was described in detail.
Domain 4: Test Model						

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Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	Perc in vitro chromosomal aberration
HERO ID:	7768

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	Test models were described in detail and appropriate for the endpoints assessed.
	Metric 15:	Number per Group	Low	× 1	3	There was only one study group for each of the three exposure concentrations tests (i.e., no replicates).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The assessment methodology addressed the intended outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment protocol was consistent across study groups.
	Metric 18:	Sampling Adequacy	Medium	× 2	4	The number of cells/dose (100) was reported and is slightly less than appropriate.
	Metric 19:	Blinding of Assessors	High	× 1	1	Test substance was supplied under code; assessors did not know its identity until after scoring.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	There were no confounding variables in test design or procedures that were reported by study authors.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	There were no confounding variables reported unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analyses were clearly described and presented in results tables.
	Metric 23:	Data Interpretation	High	× 2	2	Data were reported in such a way as to allow interpretation of test results.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints such as induction of cell death and delay in cell cycle progression were noted, and selected exposure doses were based on relation to toxicity. However, methods of measurement for specific cytotoxicity endpoints were not described.
	Metric 25:	Reporting of Data	High	× 2	2	Data were presented for percent cells with aberrations in three ways for each exposure concentration: total, simple, and complex aberrations.
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	S. M. Galloway, M. J. Armstrong, C. Reuben, S. Colman, B. Brown, C. Cannon, A. D. Bloom, F. Nakamura, M. Ahmed, S. Duk, J. Rimpo, B. H. Margolin, M. A. Resnick, B. Anderson, E. Zeiger (1987). Chromosome aberrations and sister chromatid exchanges in Chinese hamster ovary cells: evaluations of 108 chemicals Environmental and Molecular Mutagenesis, 10(Suppl. 10,Suppl. 10), 1-175
Data Type:	Perc in vitro chromosomal aberration
HERO ID:	7768

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 38: **In vitro evaluation results of Callen et al 1980 for S. cerevisiae mutagenicity study**

Study Citation:	D. F. Callen, C. R. Wolf, R. M. Philpot (1980). Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in Saccharomyces cerevisiae Mutation Research, 77(1,1), 55-63
Data Type:	S. cerevisiae mutagenicity for Perc
HERO ID:	10054

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as tetrachloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity of the test substance was not reported. It was noted that the test substance contained 0.01% thymol as a stabilizer.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to the study design. The test substances used in the study exhibited dose-related increased frequencies of gene mutations (indicative of effective assay conditions).
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were adequately described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Test substance preparation was reported; methods took into account the volatility of the test substance (i.e., the use of screw-capped centrifuge tubes). Test substance storage was not reported, but this omission is unlikely to substantially impact the study results (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate (based on observations of positive responses). Preliminary experiments were used as an aid to determine the appropriate exposure time.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The study used three exposure groups plus controls, and substantial toxicity was observed at the highest tested dose (leaving only two analyzable concentrations).

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Study Citation:	D. F. Callen, C. R. Wolf, R. M. Philpot (1980). Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in Saccharomyces cerevisiae Mutation Research, 77(1,1), 55-63
Data Type:	S. cerevisiae mutagenicity for Perc
HERO ID:	10054

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	The study used two exposure groups plus controls, and substantial toxicity was observed at the highest tested dose (leaving only one analyzable concentration).
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity, source, and relevant genetic details for the various strains of S. cerevisiae were reported and appropriate for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	At least 5 plates were used per treatment condition.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest. The methods used permitted the detection of gene revertants, gene conversion, and mitotic recombinants.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to this study design.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences among treatment group parameters were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	Data on outcome differences unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analyses are not required by study type (data for individual plates were pooled, so that independent statistical analyses are not possible). Data were presented as the number of revertants, recombinants, or convertants per 10^5 survivors (pooled data); data for numbers of revertants, recombinants, or convertants per plate (and including a measure of variation) were not reported.
	Metric 23:	Data Interpretation	High	× 2	2	The criteria for a positive result was explicitly specified (i.e., at least a doubling of colonies compared to the controls).
	Metric 24:	Cytotoxicity Data	High	× 1	1	A measure of cytotoxicity (percent survival compared to control, measured by total number of colonies counted) was determined concurrently with the mutagenicity assay results.

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Study Citation:	D. F. Callen, C. R. Wolf, R. M. Philpot (1980). Cytochrome P-450 mediated genetic activity and cytotoxicity of seven halogenated aliphatic hydrocarbons in Saccharomyces cerevisiae Mutation Research, 77(1,1), 55-63
Data Type:	S. cerevisiae mutagenicity for Perc
HERO ID:	10054

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	Data were reported 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 39: **In vitro evaluation results for Bartsch et al 1979 for mutagenicity study**

Study Citation:	H. Bartsch, C. Malaveille, A. Barbin, G. Planche (1979). Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues: Evidence for oxirane formation by P450-linked microsomal mono-oxygenases Archives of Toxicology, 41(4,4), 249-277
Data Type:	Mutagenicity for Perc
HERO ID:	10689

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 (tetrachloroethylene). A structure was also provided.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified (Merck, Darmstadt, FRG). Although a lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported (99.7%). The test substance purity was high enough that any observed effects were highly likely to be due to the nominal test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using a concurrent negative vehicle (DMSO) control group.
	Metric 5:	Positive Controls	Medium	× 2	4	The study noted that "the mutability of the strains was checked with methylmethane sulphonate and N-methyl-N'-nitro-N-nitroso-guanidine". These positive controls did not appear to have been conducted concurrently. However, some test substances did show a dose-dependent response, so it is apparent that a positive response was able to be detected.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were well-described (e.g., test conditions and incubation temperatures).
	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	Test substance preparation/storage conditions were not described in detail; however, this would not be expected to have a substantial impact on the results given that it is a short-term study.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported. It is inferred from the text that exposure administration was consistent across treatment groups.

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Study Citation:	H. Bartsch, C. Malaveille, A. Barbin, G. Planche (1979). Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues: Evidence for oxirane formation by P450-linked microsomal mono-oxygenases Archives of Toxicology, 41(4,4), 249-277
Data Type:	Mutagenicity for Perc
HERO ID:	10689

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	Exposure concentrations were not reported. It was only reported that concentrations up to 4E-3 M were tested and that concentrations above 5E-4 were toxic.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration (48 hr direct plate incorporation method) was reported and appropriate.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	Exposure groups and dose spacing was not reported.
	Metric 13:	Metabolic Activation	Low	× 1	3	The presence of a metabolic activation system was reported in the study, but not validated (mice treated with phenobarbital only rather than PB and beta-naphthoflavone). The study indicated that bacteria were exposed to the test substance in the presence of liver S9 and in the presence or absence of "cofactors" (NADP+ and glucose 6-phosphate). There was no indication that tests were carried out in the absence of metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The source of the test model (bacterial strains) was reported (i.e., provided by Professor Ames) and the model is the most commonly used for this type of assay. It was indicated that the presence of an R factor was tested (by seeding on plates containing ampicillin); mutability of the strains was also checked.
	Metric 15:	Number per Group	High	× 1	1	The number of replicates per group were reported and appropriate for the study type (triplicate plating).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the intended outcome of interest (number of revertants/plate).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	It was inferred from the text that the endpoint of interest was assessed consistently.
	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	Blinding was not addressed and is not considered appropriate for the study type.
Domain 6: Confounding / Variable Control						

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Study Citation:	H. Bartsch, C. Malaveille, A. Barbin, G. Planche (1979). Mutagenic and alkylating metabolites of halo-ethylenes, chlorobutadienes and dichlorobutenes produced by rodent or human liver tissues: Evidence for oxirane formation by P450-linked microsomal mono-oxygenases Archives of Toxicology, 41(4,4), 249-277
Data Type:	Mutagenicity for Perc
HERO ID:	10689

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among study group parameters (e.g., test substance, bacterial strain used) that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variable unrelated to exposure were reported or identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	No statistical analysis was conducted. No raw data (means, standard deviations) were provided, so independent statistical analysis is not possible. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay, so this is still considered acceptable.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were partially reported in the results. The results report dose-related and/or 2-fold increases in revertant frequency as indicative of a positive response; however, criteria were not explicitly specified (and a less than 2-fold response was indicated as positive).
	Metric 24:	Cytotoxicity Data	High	× 1	1	The absence of a background lawn of bacteria was used as an indication of gross toxicity. Toxicity was noted at 5E-4 M Perc and above (tested up to 4E-3 M Perc).
	Metric 25:	Reporting of Data		× 2	NA	Data were reported qualitatively.
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 40: **In vitro evaluation results for Tu et al 1985 for transformation assay in mouse embryo cells**

Study Citation:	A. S. Tu, T. A. Murray, K. M. Hatch, A. Sivak, H. A. Milman (1985). In vitro transformation of BALB/c-3T3 cells by chlorinated ethanes and ethylenes Cancer Letters, 28(1,1), 85-92
Data Type:	In vitro transformation assay for perc
HERO ID:	17978

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as tetrachloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified (purchased from Aldrich Chemical Company and provided by Dr. Mitoma of SRI International). Although a lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The purity of the test substance was not explicitly specified; however, it was indicated that the purity of all test chemicals was 97% to 99%. Therefore, the purity was such that observed effects were more likely than not due to the nominal test substance.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The use of a concurrent (untreated) control group was reported.
	Metric 5:	Positive Controls	High	× 2	2	A concurrent positive control was used and the intended positive result was induced. All plates treated with 3-methylcholanthrene (MCA) had type III foci (an acceptable level of transformation was observed).
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures (for the standard assay) were briefly described and partially cited to another publication (Sivak and Tu 1980), but appeared adequate for the endpoint of interest.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Not Rated	NA	NA	Information on preparation and storage were not reported; standard assay procedures were cited to another publication (Sivak and Tu 1980). It is noted that the test substance was not treated as one of the "more volatile" chemicals in the study (and therefore, standard rather than modified procedures were used).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.

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Study Citation:	A. S. Tu, T. A. Murray, K. M. Hatch, A. Sivak, H. A. Milman (1985). In vitro transformation of BALB/c-3T3 cells by chlorinated ethanes and ethylenes Cancer Letters, 28(1,1), 85-92
Data Type:	In vitro transformation assay for perc
HERO ID:	17978

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations of the test substance were reported without ambiguity in Table 1.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration (3 days) was reported and is appropriate for the study type/outcome of interest (cell transformation).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups (4 doses plus controls) was reported. Substantial cytotoxicity was evident at the high dose (surviving fraction = 4%). No rationale for the selection of these doses was provided (the toxicity test appeared to be concurrent rather than preliminary).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to this study type. Cell transformation assays may be conducted in the presence of activation, but is not a requirement by study type.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (BALB/c-3T3 cells) and descriptive information (origin = NCI; taken from stock and not maintained beyond first passage) were reported, and the test model is routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	The total plates per dose group for Perc was 19-20. (Reference to duplicate plates is in regards to cell counts for the cytotoxicity assessment.) This is considered appropriate for the study type and outcome analysis.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology used addressed the intended outcomes of interest (foci with Type III characteristics).
	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 (approximately 30 days after exposure).
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this study type (all foci were scored).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not mentioned in the study report; therefore; this metric is considered not applicable to this study type.
Domain 6: Confounding / Variable Control						

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Study Citation:	A. S. Tu, T. A. Murray, K. M. Hatch, A. Sivak, H. A. Milman (1985). In vitro transformation of BALB/c-3T3 cells by chlorinated ethanes and ethylenes Cancer Letters, 28(1,1), 85-92
Data Type:	In vitro transformation assay for perc
HERO ID:	17978

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among study group parameters (e.g., test substance, cells used) that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variable unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical significance is referenced in the discussion of results for another test compound, but no details regarding the type of statistical test conducted were included. However, data were sufficient data to conduct an independent statistical analysis (based on mean numbers of type III foci/plate and plates with Type III foci/total plates).
	Metric 23:	Data Interpretation	Medium	× 2	4	The study authors reported the scoring criteria (characteristics of scored Type III foci) for the test; these characteristics, which were consistent with established practices, which were partially cited to another publication (Reznikoff et al., 1973).
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints were defined and methods of measurement were partially reported, but the omissions are unlikely to have substantial impact on study results.
	Metric 25:	Reporting of Data	Medium	× 2	4	Data for exposure-related findings were presented for all outcomes by exposure group. In Table 1, the test substance is not indicated; it is inferred from text that results are relevant to tetrachloroethylene.
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 41: **In vitro evaluation results of Haworth et al 1983 for bacterial reverse mutation study**

Study Citation:	S. Haworth, T. Lawlor, K. Mortelmans, W. Speck, E. Zeiger (1983). Salmonella mutagenicity test results for 250 chemicals Environmental Mutagenesis, 5(Suppl 1,Suppl 1), 3-142
Data Type:	Bacterial reverse mutation for Perc
HERO ID:	28947

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as tetrachloroethylene with the correct CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported, including manufacturer lot number.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be "Technical" grade according to the manufacturer label.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative control groups were included (DMSO).
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were tested concurrently with each test substance. The identity of each positive control was reported and appropriate for different strains with and without metabolic activation. Positive controls yielded positive results.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described in detail and were applicable to the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is 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 reported. Test substance storage was not reported (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration for the pre-incubation protocol was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The maximum dose was chosen based on solubility limits or cytotoxicity. The number of exposure groups and dose spacing was reported and appropriate for this assay (3.3, 10, 33, 100, or 333 µg/plate).
	Metric 13:	Metabolic Activation	Medium	× 1	2	The source and method of preparation of the rat liver S9 fraction was reported; however, the concentration of S9 in the bacterial mutagenicity assay was not specified.

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Study Citation: S. Haworth, T. Lawlor, K. Mortelmans, W. Speck, E. Zeiger (1983). Salmonella mutagenicity test results for 250 chemicals Environmental Mutagenesis, 5(Suppl 1,Suppl 1), 3-142
Data Type: Bacterial reverse mutation for Perc
HERO ID: 28947

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity and donor source of the bacterial strains used here were identified, and these strains are routinely used for the outcome of interest. It was noted that the cultures were "routinely checked for genetic integrity as recommended by Ames et al. (1975)."
	Metric 15:	Number per Group	High	× 1	1	Each assay was plated in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Number of colonies is an objective outcome and blinding assessors is not necessary; however, the identity of each test substance assessed in this study was coded and not known to the assessors.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were 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.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	A positive result was defined as a "reproducible, dose-related increase, whether it be twofold over background or not." Therefore, no statistical analysis was reported directly in the study; however, this is appropriate for this study design. Raw data are provided and could be analyzed independently.
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria (number of colonies) was reported and consistent with current standards.
	Metric 24:	Cytotoxicity Data	High	× 1	1	A dose-setting experiment was conducted to assess cytotoxicity levels (viability, reduced numbers of colonies). If toxicity was observed in the preliminary experiment, the doses for the mutagenicity assay were selected so that the highest dose exhibited some degree of toxicity.

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Study Citation:	S. Haworth, T. Lawlor, K. Mortelmans, W. Speck, E. Zeiger (1983). Salmonella mutagenicity test results for 250 chemicals Environmental Mutagenesis, 5(Suppl 1,Suppl 1), 3-142
Data Type:	Bacterial reverse mutation for Perc
HERO ID:	28947

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	All data are adequately 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 42: **In vitro evaluation results for Price et al 1978 for cell transformation assay in rat embryo cells**

Study Citation:	P. J. Price, C. M. Hassett, J. I. Mansfield (1978). Transforming activities of trichloroethylene and proposed industrial alternatives In Vitro, 14(3,3), 290-293					
Data Type:	Cell transformation assay for perc					
HERO ID:	29449					
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 (tetrachloroethylene; TTCl).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was identified (Eastman Kodak). Although batch/lot numbers were not provided, the test substance is not expected to vary in composition.
	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	Medium	× 2	4	The study authors reported using a concurrent negative control group, but all conditions were not equal to those of treated groups. However, the identified differences are considered to be minor limitations that are unlikely to have substantial impact on results. It is indicated that the negative control was acetone at a concentration of 1:1000; the positive control was also diluted in acetone. The study does not state that the test substance was diluted in acetone. However, an additional medium only group was used.
	Metric 5:	Positive Controls	Medium	× 2	4	A concurrent positive control was used, and is appropriate for the study type (i.e., cell transformation assays). The results indicate that the positive control induced transformation; however, the response not further characterized, and appeared to be similar in magnitude to the response for the test substance(s).
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures (e.g., test conditions, cell density, culture media, and volumes) were described in adequate detail.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation/storage conditions were not described in detail (other than the test substance has a half-life > 2 years); however, this would not be expected to have a substantial impact on the results given that it is a short-term study.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were administered consistently across study groups.

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Study Citation:	P. J. Price, C. M. Hassett, J. I. Mansfield (1978). Transforming activities of trichloroethylene and proposed industrial alternatives In Vitro, 14(3,3), 290-293
Data Type:	Cell transformation assay for perc
HERO ID:	29449

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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 exposure duration (48 hours) was reported and appears to be appropriate for the study type/outcome of interest (cell transformation).
	Metric 12:	Exposure Route and Method	Low	× 1	3	There were deficiencies regarding the number of exposure groups and/or concentration spacing. Only two concentrations of the test substance were tested (with no rationale for their selection).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to this study type. Cell transformation assays may be conducted in the presence of activation, but is not a requirement by study type.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was reported along with limited descriptive information (described previously in Freeman et al. 1975). Limited information regarding the cells (passage, genetic information) was provided. The source was not reported. It is not clear that this cell type (Fischer rat embryo F1706 cells) is routinely used for this study type.
	Metric 15:	Number per Group	High	× 1	1	For the transformation assay, the use of quadruplicate cultures were reported. The number of replicates per study group were reported and were considered appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Low	× 2	6	It was not clear that the outcome assessment (evidence of transformation 2 to 4 subcultures after treatment) was a sensitive measure of transformation potential.
	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 this study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding is not mentioned in the study report; therefore; this metric is considered not applicable to this study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no reported differences among study group parameters (e.g., test substance, cells used) that could influence the outcome assessment.

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Study Citation:	P. J. Price, C. M. Hassett, J. I. Mansfield (1978). Transforming activities of trichloroethylene and proposed industrial alternatives In Vitro, 14(3,3), 290-293
Data Type:	Cell transformation assay for perc
HERO ID:	29449

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variable unrelated to exposure were reported or identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Unacceptable	× 1	4	No statistical analyses were conducted (cell transformation assay) and data for average number of foci (three plates) were not provided with a measure of variation (for independent analyses). The number of plates with foci/number of plates were also not reported/could not be analyzed. There was no evidence that the positive control induced a statistically significantly increased transformation frequency.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were partially reported (e.g., characteristics of transformed foci). However, a complete description of the criteria for a positive response was not provided (transformation by the a certain subsculture and/or numbers of microscopic foci).
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints were defined and methods of measurement were partially reported. The authors indicated that a test was conducted before the transformation assay. Perc was tested only at concentrations that yielded relative plating efficiencies of 88% and 63%.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group.
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 43: **Animal toxicity evaluation results for Bronzetti et al 1983 for host-mediated genotoxicity study**

Study Citation:	G. Bronzetti, C. Bauer, C. Corsi, R. Del Carratore, A. Galli, R. Nieri, M. Paolini (1983). Genetic and biochemical studies on perchloroethylene 'in vitro' and 'in vivo' Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 116(3-4,3-4), 323-331
Data Type:	Host-mediated yeast genotoxicity
HERO ID:	58230

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 and structure.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	>99.5% pure; impurities reported as HCl, NH3, water, and residual
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	A concurrent negative control group was used, but details regarding the negative control group were not (not clear whether corn oil vehicle controls were used).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were used for the in vitro experiment, but not for the host -mediated assay.
	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	Test substance was prepared in corn oil; storage was not desribed.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure was consistent across groups and gavage volume was not excessive.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Acute and repeat dose experiments were performed. Yeast were injected 4 h before animals were sacrificed.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Single dose level per experiment.
	Metric 12:	Exposure Route and Method	High	× 1	1	Oral gavage in corn oil is appropriate for perc.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Low	× 2	6	The source of the test animal was not reported. Species, strain and sex were reported, but not age, body weight or health status.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Husbandry conditions were not reported.

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Study Citation:	G. Bronzetti, C. Bauer, C. Corsi, R. Del Carratore, A. Galli, R. Nieri, M. Paolini (1983). Genetic and biochemical studies on perchloroethylene 'in vitro' and 'in vivo' Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 116(3-4,3-4), 323-331
Data Type:	Host-mediated yeast genotoxicity
HERO ID:	58230

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	Tables indicate 5/group.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment reported and was sensitive for the outcome of interest (point mutation or mitotic recombination in yeast).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	Yeast were extracted from liver, lungs and kidney.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric was not applicable to the outcome of interest.
	Metric 20:	Negative Control Response	High	× 1	1	The negative control response appeared adequate.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	The lack of reporting of initial body weights and food/water intake is not likely to have a significant impact on results.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	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 were not performed; however mean +/- SD values were reported and an independent statistical analysis could be performed.
	Metric 24:	Reporting of Data	High	× 2	2	Data were presented for each tissue and each 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 44: **In vitro evaluation results for Kringstad et al 1981 for mutation assay in S. typhimurium**

Study Citation:	K. P. Kringstad, P. O. Ljungquist, F. de Sousa, L. M. Stromberg (1981). Identification and mutagenic properties of some chlorinated aliphatic compounds in the spent liquor from kraft pulp chlorination Environmental Science and Technology, 15(5,5), 562-566
Data Type:	in vitro mutation assay in S. typhimurium - Perc
HERO ID:	35086

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as tetrachloroethylene
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (E. Merck). The product number and batch/lot number were not reported; however, the material is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity and/or grade of the test substance was reported (99%)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors report using a vehicle control (ether)
	Metric 5:	Positive Controls	Low	× 2	6	A positive control was used (methyl methanesulfonate; however, the response of the positive control were not reported.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were briefly described, but appeared appropriate. More detailed methods were cited to other references (Ander et al., 1977).
	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	High	× 1	1	Test substance preparation was described as added in ether solution (20ul/plate).
	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	One test concentration was reported in the results without ambiguity (0.1 mg/plate)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	The exposure duration was not reported. More detailed methods were cited to other references (Ander et al., 1977).

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Study Citation:	K. P. Kringstad, P. O. Ljungquist, F. de Sousa, L. M. Stromberg (1981). Identification and mutagenic properties of some chlorinated aliphatic compounds in the spent liquor from kraft pulp chlorination Environmental Science and Technology, 15(5,5), 562-566
Data Type:	in vitro mutation assay in S. typhimurium - Perc
HERO ID:	35086

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure concentrations were not clearly reported. The study noted that the amount of single model compounds added was varied over a wide range covering survival from 1-100%, including 6-8 different (unspecified) dosage levels. Only 1 test concentration was reported in the results. There is no indication if there was toxicity at the highest dose tested. It is noted in the results that the doses presented "were about the highest possible which yield 70-100% bacterial survival for each tested compound". This metric is determined to be unacceptable due to the uncertainty of cytotoxicity at this dose.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable; the test organism, S. typhimurium was used without the addition of metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test models and source were reported and appropriate for the outcome of interest (S. typhimurium TA 1535). It is noted that it is unusual to only utilize one S. typhmurium tester strain for the bacterial reverse mutation assay; however, the single strain utilized is considered valid in itself.
	Metric 15:	Number per Group	Medium	× 1	2	Reported results were mean values of 3 or more assays. There is some uncertainty because the minimum number of replicates was reported, but the specific amount of replicates for each treatment group was not reported. However, 3 assays is considered sufficient for the outcome of interest.
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 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

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Study Citation:	K. P. Kringstad, P. O. Ljungquist, F. de Sousa, L. M. Stromberg (1981). Identification and mutagenic properties of some chlorinated aliphatic compounds in the spent liquor from kraft pulp chlorination Environmental Science and Technology, 15(5,5), 562-566
Data Type:	in vitro mutation assay in S. typhimurium - Perc
HERO ID:	35086

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variable unrelated to exposure were reported or identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistics were not used to assess increased revertants/plate from the control. It was noted that the compound was listed positive when the number of revertants exceeded the background level by a factor of 2 or more. Only means (with no measure of variance, e.g. standard deviation; and no specific number of replicates) were included in the results so independent statistical analysis could not be performed. Statistical analysis is not necessarily required for the bacterial reverse mutation assay, so the data analysis is considered acceptable.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria were reported and appropriate.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoints and methods were described (cell death)
	Metric 25:	Reporting of Data	Low	× 2	6	Data for the outcome was presented; however, data were not shown for each study group, data for the positive control and cytotoxicity data were not reported.
Overall Quality Determination‡			Unacceptable⋆⋆		1.5	
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 45: **In vitro evaluation results for Greim et al 1975 for bacterial mutagenicity study**

Study Citation:	H. Greim, G. Bonse, Z. Radwan, D. Reichert, D. Henschler (1975). Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation Biochemical Pharmacology, 24(21,21), 2013-2017
Data Type:	Mutagenicity of E. coli - Perc
HERO ID:	58073

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Tetrachloroethylene was identified by chemical name and structure (Table 1).
	Metric 2:	Test Substance Source	High	× 1	1	Obtained from Merc & Co., Darmstadt.
	Metric 3:	Test Substance Purity	High	× 1	1	Chemicals from this source were obtained as a.g. reagents.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	The study authors did not report the use of a concurrent negative control group.
	Metric 5:	Positive Controls	Medium	× 2	4	A positive control group was not reported, but vinyl chloride was concurrently tested and the authors reported it produced positive responses with metabolic activation, indicating the test system was capable of detecting a positive response (although the evaluation criteria for a positive response was not specified).
	Metric 6:	Assay Procedures	Medium	× 1	2	Test methods/procedures were briefly described or were cited to another source (C. Mohn, et al. 1974), but appeared appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The study only reports varying concentrations of 5 uL of the liquid test substance were added (injected) to the medium. No other preparation details were provided. The pre-incubation method was used and appropriate for the test substances. No storage details were required due to the short study duration (2 hours).
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposure appears consistent across the study groups; however, it is not specifically stated. Methods were briefly described or cited elsewhere.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Tetrachloroethylene was tested at 0.9 nM.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was 2 hours and was appropriate for this study type.

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Study Citation:	H. Greim, G. Bonse, Z. Radwan, D. Reichert, D. Henschler (1975). Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation Biochemical Pharmacology, 24(21,21), 2013-2017
Data Type:	Mutagenicity of E. coli - Perc
HERO ID:	58073

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	One concentration was used on one bacterial strain (E. coli K12) with 4 different operons (gal+, arg+, MTR, and nad+). Cell survival was 99% for tetrachloroethylene. the study notes that the test concentrations were chosen based on the results of a preliminary experiment in order to not reduce cell survival by >20%. No additional details of the preliminary experiment results were provided.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The study reports cells were exposed both with and without metabolic activation. 5 mg of liver microsomes from male mice pretreated with 0.1% phenobarbital in drinking water for 10 days were used as the metabolic activation. Method of preparation was not reported.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	E. coli K12 was used in this experiment with 4 different operons (gal+, arg+, MTR, and nad+). It is unclear if this strain was from a commercial source or laboratory-maintained. No other strains were tested in a mutagenicity test.
	Metric 15:	Number per Group	Low	× 1	3	The number of replicates used in this study was not specified, but it is assumed as a single assay.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Mutagenicity was evaluated by counting the number of colony-forming units on the selective media per the number of colony-forming units on the complete medium, presented as the % spontaneous mutation rate (Table 1). Cytotoxic concentrations were deliberately avoided based on the results of the preliminary test.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	No inconsistencies were reported, and consistency appeared appropriate. However, details results in the absence of metabolic activation were not provided.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design (mutagenicity assay).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design, as no subjective outcomes were assessed.
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:	H. Greim, G. Bonse, Z. Radwan, D. Reichert, D. Henschler (1975). Mutagenicity in vitro and potential carcinogenicity of chlorinated ethylenes as a function of metabolic oxirane formation Biochemical Pharmacology, 24(21,21), 2013-2017
Data Type:	Mutagenicity of E. coli - Perc
HERO ID:	58073

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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	Not Rated	NA	NA	Statistical analysis was not performed, and although individual results were provided in Table 1 in the presence of metabolic activation, no negative control was used and a dose-response analysis is not possible because only 1 concentration was tested. Results in the absence of metabolic activation were generally summarized as negative and no individual data was provided. However, statistical analysis is not necessarily required for the bacterial reverse mutation assay.
	Metric 23:	Data Interpretation	Low	× 2	6	The scoring and/or evaluation criteria was not described, and it is unclear how a positive result was determined.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The percent survival of bacteria on the full media was reported, and the chosen concentration was based on the cytotoxicity results from a preliminary test, with a goal of <20% cell death.
	Metric 25:	Reporting of Data	High	× 2	2	Individual results were reported for in Table 1 in the presence of metabolic activation. All chemicals tested (6 total) were reported as negative for mutagenicity in the absence of metabolic activation (individual results not reported).
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 =≥ 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 46: **In vitro evaluation results for Bronzetti et al 1983 for genotoxicity study**

Study Citation:	G. Bronzetti, C. Bauer, C. Corsi, R. Del Carratore, A. Galli, R. Nieri, M. Paolini (1983). Genetic and biochemical studies on perchloroethylene 'in vitro' and 'in vivo' Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 116(3-4,3-4), 323-331
Data Type:	genotoxicity in yeast
HERO ID:	58230

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by chemical name and structure.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified.
	Metric 3:	Test Substance Purity	High	× 1	1	99.5% pure with impurities identified (HCl, NH3, water, residual).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Negative controls were used, but it is not clear whether these represented vehicle controls (no details were provided).
	Metric 5:	Positive Controls	High	× 2	2	Dimethylnitrosamine (DMNA) was used as a positive control.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and cited in another publication (Zimmerman, 1973), but appeared to be appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The test substance was prepared in DMSO. Storage was not described, but this is appropriate given the study design (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure was administered consistently across groups (0.1ml in DMSO).
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported in units of mM.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	Suspensions were incubated for 2h. Positive control was responsive at this duration.
	Metric 12:	Exposure Route and Method	High	× 1	1	5 concentrations were used; highest concentration had low survival.
	Metric 13:	Metabolic Activation	High	× 1	1	Exposures were conducted in the presence and absence of metabolic activation and the type and source, method of preparation, concentration or volume in final solution was described.
Domain 4: Test Model						

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Study Citation:	G. Bronzetti, C. Bauer, C. Corsi, R. Del Carratore, A. Galli, R. Nieri, M. Paolini (1983). Genetic and biochemical studies on perchloroethylene 'in vitro' and 'in vivo' Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 116(3-4,3-4), 323-331
Data Type:	genotoxicity in yeast
HERO ID:	58230

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported with limited descriptive information and was routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	5 replicates were used per concentration.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methods reported and were sensitive for the outcome of interest (mitotic gene conversion at the trp locus, mitotic recombination between the centromere and the ade2 locus and point mutation at the ilv) .
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	initial conditions were the same across groups (1.0 ml of cell suspension; 6 x 10ˆ8 cells/ml buffer).
	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	Statisics were not performed, but mean +/- SD values were reported allowing for independent statistical analysis.
	Metric 23:	Data Interpretation	Low	× 2	6	Scoring and/or evaluation criteria were not reported.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were defined as % survival, but the methods of measurements were not fully described or reported.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for each treatment group.
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

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Study Citation:	G. Bronzetti, C. Bauer, C. Corsi, R. Del Carratore, A. Galli, R. Nieri, M. Paolini (1983). Genetic and biochemical studies on perchloroethylene 'in vitro' and 'in vivo' Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 116(3-4,3-4), 323-331
Data Type:	genotoxicity in yeast
HERO ID:	58230

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 47: **In vitro evaluation results for Kline et al 1982 for bacterial mutagenicity study**

Study Citation:	S. A. Kline, E. C. Mccoy, H. S. Rosenkranz, B. L. Van Duuren (1982). Mutagenicity of chloroalkene epoxides in bacterial systems Mutation Research, 101(2,2), 115-125
Data Type:	in vitro mutation assay in S. typhimurium and E. coli- Perc Oxide
HERO ID:	58237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as tetrachloroethylene oxide (chemical structure provided)
	Metric 2:	Test Substance Source	Unacceptable	× 1	4	Analytical verification of the synthesized test substance was not conducted.
	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 both untreated and vehicle controls (acetone).
	Metric 5:	Positive Controls	High	× 2	2	Appropriate positive controls were used (AF-2 for E.coli and NaN3 for S. typhimurium) in the mutagenicity assay.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were briefly described but appeared appropriate. More detailed methods were cited to other references (McCoy et al., 1978 for mutagenicity assay and Hyman et al., 1980 for the DNA-repair assay).
	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	Low	× 1	3	Test substance preparation was described as diluted in acetone (10ul dilutions); The storage of the test substance was not reported. This is likely to have affected results, given that the half life of tetrachloroethylene-oxide was reported to be 11.5 minutes in water. It is likely that the lack of reported test substance storage substantially affected results.
	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 Perc-oxide 925, 5, 2.5, 1.3, 0.5 mM)

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Study Citation:	S. A. Kline, E. C. Mccoy, H. S. Rosenkranz, B. L. Van Duuren (1982). Mutagenicity of chloroalkene epoxides in bacterial systems Mutation Research, 101(2,2), 115-125
Data Type:	in vitro mutation assay in S. typhimurium and E. coli- Perc Oxide
HERO ID:	58237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration was reported (20 minutes). It is noted that given a half life of 11.5 minutes, it would be expected that 29.96% of the original amount of the test substance would be present in solution after 20 minutes.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure concentrations were reported. The number of exposure groups and spacing of exposure levels were not justified, but were adequate to show results relevant to the outcome of interest
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable; the test organism, TCE and Perc metabolites were tested without the addition of metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test models were reported with some descriptive information and appropriate for the outcome of interest; the source of the bacteria Mutation assay: S. typhimurium 1535 and E. coli WP2uvrA
	Metric 15:	Number per Group	High	× 1	1	3 replicates per treatment group is considered adequate.
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 method is not applicable to the outcome.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial strain/batch/lot number of organisms or models used per group, size, and/or quality of tissues exposed was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						

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Study Citation:	S. A. Kline, E. C. Mccoy, H. S. Rosenkranz, B. L. Van Duuren (1982). Mutagenicity of chloroalkene epoxides in bacterial systems Mutation Research, 101(2,2), 115-125
Data Type:	in vitro mutation assay in S. typhimurium and E. coli- Perc Oxide
HERO ID:	58237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Medium	× 1	2	Statistics were not used to assess increased revertants/plate from the control. Means (with standard deviation) were included in the results so independent statistical analysis may be performed. 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	The evaluation criteria were reported to be exhibiting toxicity, as evidenced by a decrease in the spontaneous frequency of the revertants and/or by an inhibition of the growth of the bacteria; evaluation of mutagenic potential was not described.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoints were described (decreased spontaneous frequency of revertants)
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcomes were presented for each exposure groups, including negative and positive controls
Overall Quality Determination‡			Unacceptable⋆⋆		1.7	
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 48: **In vitro evaluation results for Kline et al 1982 for bacterial DNA repair study**

Study Citation:	S. A. Kline, E. C. Mccoy, H. S. Rosenkranz, B. L. Van Duuren (1982). Mutagenicity of chloroalkene epoxides in bacterial systems Mutation Research, 101(2,2), 115-125
Data Type:	DNA-repair assay in E. coli - Perc-oxide
HERO ID:	58237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as tetrachloroethylene oxide (chemical structure provided)
	Metric 2:	Test Substance Source	Unacceptable	× 1	4	Analytical verification of the synthesized test substance was not conducted.
	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 vehicle control (acetone).
	Metric 5:	Positive Controls	High	× 2	2	An appropriate positive control was used (ethyl methanesulfonate for the DNA-repair assay).
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were briefly described but appeared appropriate. More detailed methods were cited to other references (McCoy et al., 1978 for mutagenicity assay and Hyman et al., 1980 for the DNA-repair assay).
	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	Low	× 1	3	Test substance preparation was described as diluted in acetone (10ul dilutions); The storage of the test substance was not reported. This is likely to have affected results, given that the half life of tetrachloroethylene-oxide was reported to be 11.5 minutes in water. It is likely that the lack of reported test substance storage substantially affected results.
	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 Perc-oxide (0.44, 0.09, 0.04 uM/ml)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration was reported (20 minutes). It is noted that given a half life of 11.5 minutes, it would be expected that 29.96% of the original amount of the test substance would be present in solution after 20 minutes.

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Study Citation: S. A. Kline, E. C. Mccoy, H. S. Rosenkranz, B. L. Van Duuren (1982). Mutagenicity of chloroalkene epoxides in bacterial systems Mutation Research, 101(2,2), 115-125
Data Type: DNA-repair assay in E. coli - Perc-oxide
HERO ID: 58237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure concentrations were reported. The number of exposure groups and spacing of exposure levels were not justified, but were adequate to show results relevant to the outcome of interest
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable; the test organism, TCE and Perc metabolites were tested without the addition of metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test models were reported with some descriptive information and appropriate for the outcome of interest; The source of the bacteria was not reported DNA-repair assay: E. coli polA1+ and E. coli polA1-
	Metric 15:	Number per Group	Medium	× 1	2	2 replicates per treatment group is considered somewhat lacking.
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 method is not applicable to the outcome.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial strain/batch/lot number of organisms or models used per group, size, and/or quality of tissues exposed was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	Results for the DNA-repair assay are expressed as % survival compared to control. This was based on an average (of 2 plates) colonies/plate (variance was not reported) for each test concentration. A survival index (% survival polA1+/%survival pol A1+) was also reported. Statistical analysis is not necessarily required for this assay, so the data analysis is considered acceptable.

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Study Citation:	S. A. Kline, E. C. Mccoy, H. S. Rosenkranz, B. L. Van Duuren (1982). Mutagenicity of chloroalkene epoxides in bacterial systems Mutation Research, 101(2,2), 115-125
Data Type:	DNA-repair assay in E. coli - Perc-oxide
HERO ID:	58237

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria were reported and appropriate (Survival index values below 0.85 indicated preferential inhibition of polA-)
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoints were described (decreased spontaneous frequency of revertants)
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcomes were presented for each exposure groups, including negative and positive controls
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 49: **In vitro evaluation results for Beliles et al 1980 for unscheduled DNA synthesis study**

Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	PERC UDS
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chemical was identified by name and CAS
	Metric 2:	Test Substance Source	High	× 1	1	Source was reported, North Strong, and analytically verfied
	Metric 3:	Test Substance Purity	Medium	× 1	2	analyzed 91.43% purity, impurities were not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Solvent control was reported
	Metric 5:	Positive Controls	High	× 2	2	MNNG and BaP were reported as positive con trols -/+ S9, respectively.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedure was partially reported and appeared appropriate for the study type.
	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	Medium	× 1	2	Test substance was prepared in DMSO solvent and cell medium.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was assumed to be consistent across all study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Test concentrations range 0., 0.5, 1.0, 5.015.0 ug/mL (reports ul/ml in results but can be converted).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	Exposure duration was 1.5h, less than recommended but only slightly.
	Metric 12:	Exposure Route and Method	High	× 1	1	Concentrations were 3 doses and controls and spacing was based on cytotoxicity seen at the high dose and appeared to be .
	Metric 13:	Metabolic Activation	High	× 1	1	metabolic activation S9 was reported
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model is reported human diploid WI-38 cells and is appropriate for the study
	Metric 15:	Number per Group	Low	× 1	3	Cell number per group was not reported but was described as confluent
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was adequate for the outcome of interest

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Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	PERC UDS
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Exposure assessment is assumed to be consistent across study groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Cell number counted/slides were not reported but was done with spec and is inferred to be autocounted
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for 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.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analysis was not reported due to lack of replicates
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria was reported as 150% or greater than controls, and appears to be appropriate.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints were previously cited, cell growth, and instances were reported at the high dose.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all outcomes and doses
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 50: **In vitro evaluation results for Beliles et al 1980 for host-mediated assay in mice**

Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	PERC host mediated assay TA98 in CD-1 mice
HERO ID:	58331

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Chemical was identified by name and CAS
	Metric 2:	Test Substance Source	High	× 1	1	Source was reported, North Strong, and analytically verfied
	Metric 3:	Test Substance Purity	Medium	× 1	2	Analyzed 91.43% purity, impurities were not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Filtered air control animals
	Metric 5:	Positive Controls	Low	× 2	6	2-aminoanthracene was used as a positive control specifically for TA98 frameshift, but gives variable results; dimethylnitrosamine was used as a second positive control for TA 1535
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were reported, however the collection of peritoneal fluid from 5 animals was mistakenly pooled, rather than analyzed individually and deviates from standard practice
	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	Method and equipment used to generate the test substance as a vapor were reported and appropriate.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was assumed to be consistent across all study groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were 100 and 500 ppm
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	Exposure duration of indicator in organism was 3 h following animal exposure (5d)
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Number of exposure groups was reported, 2, and appeared adequate, spacing was not justified
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable to the study type
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model was reported, TA98 indicator in CD-1 host, and is routinely used for the outcome of interest.

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Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	PERC host mediated assay TA98 in CD-1 mice
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	Bacterium were cultured to 1x 10ˆ10 cells/ml with 1ml injected and was appropriate for the study
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology was adequate for the outcome of interest
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	IP injection time of the indicator into host was not reported and unclear if consistent between groups (but within 2h after exposure)
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Cell number counted/slides were not reported but was done with spec and is inferred to be autocounted
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for 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.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was not reported but data was sufficient for independent analysis
	Metric 23:	Data Interpretation	High	× 2	2	Evaluation criteria was reported as greater than 2 fold the control value and appears appropriate for the study
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	not applicable for the study type
	Metric 25:	Reporting of Data	High	× 2	2	Data is reported qualitatively in table 79 and quantitatively (pooled samples of 5) in table 80
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 51: **In vitro evaluation results for Reichert et al 1983 for bacterial mutagenicity study**

Study Citation:	D. Reichert, T. Neudecker, U. Spengler, D. Henschler (1983). Mutagenicity of dichloroacetylene and its degradation products trichloroacetyl chloride, trichloroacryloyl chloride and hexachlorobutadiene Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 117(1-2,1-2), 21-29
Data Type:	Bacterial mutagenicity (Perc metabolite)
HERO ID:	59258

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 (trichloroacetyl chloride).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (Merck). Although a lot/batch number was not provided, the test substance is not expected to vary in composition.
	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	It is inferred from the text/Figure 4 that a concurrent negative control group was used; presumably, all conditions were equal except exposure to the test substance.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Although a concurrent positive control group was not used, the study indicates that the tester strains were "routinely checked" with 2-aminoathracene as a standard mutagen in the presence of activation, and 4-nitro-o-phenylenediamine (TA 98) or sodium azide (TA 100) in the absence of activation. In addition, the response for other chemicals tested in this study were positive and/or exposure-related.
	Metric 6:	Assay Procedures	High	× 1	1	Methods and procedures were described in adequate detail (e.g., temperatures, cell density, and test conditions).
	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 preparation of the test substance was described in minimal detail (liquid suspension system cited to Rannug et al. 1976). The study indicated that the test substance was diluted in acetonitrile; tubes were tightly closed using screw caps during the incubation period. Given the short-term nature of the experiment, omissions with respect to storage conditions are not likely to substantially impact the study results.

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Study Citation:	D. Reichert, T. Neudecker, U. Spengler, D. Henschler (1983). Mutagenicity of dichloroacetylene and its degradation products trichloroacetyl chloride, trichloroacryloyl chloride and hexachlorobutadiene Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 117(1-2,1-2), 21-29
Data Type:	Bacterial mutagenicity (Perc metabolite)
HERO ID:	59258

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures appeared to be consistently applied across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were not explicitly specified, but could be estimated based on data present in Figure 4.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was appropriate for the outcome of interest (as evidenced by increased number of revertants in some assays/for some chemicals).
	Metric 12:	Exposure Route and Method	High	× 1	1	Five analyzable concentrations of the test substance were tested.
	Metric 13:	Metabolic Activation	High	× 1	1	The study authors reported using a metabolic activation system; the source and preparation of S9 was reported.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (S.typhmurium strains) are commonly used for assays of this type. Strains TA 98 and TA 100 were obtained from a laboratory-maintained culture (Ames laboratory).
	Metric 15:	Number per Group	High	× 1	1	Duplicate plates were used at each exposure concentration.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment partially addressed the outcome of interest (reverse mutation in the absence of cytotoxicity).
	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	Low	× 2	6	Initial conditions were not reported for each group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						

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Study Citation:	D. Reichert, T. Neudecker, U. Spengler, D. Henschler (1983). Mutagenicity of dichloroacetylene and its degradation products trichloroacetyl chloride, trichloroacryloyl chloride and hexachlorobutadiene Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 117(1-2,1-2), 21-29
Data Type:	Bacterial mutagenicity (Perc metabolite)
HERO ID:	59258

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analyses are not required by study type. However, data for the study (S.typhmurium strain TA 100) were presented graphically (but without a measure of variation). Data could be analyzed independently by evaluating the increase in the mean number of revertants relative to controls.
	Metric 23:	Data Interpretation	Medium	× 2	4	It was inferred from the text that the criteria for a positive result was a concentration-related increased number of revertants (although this was not explicitly specified).
	Metric 24:	Cytotoxicity Data	High	× 1	1	The methods used to assess cytotoxicity were reported; cytotoxicity data were presented (graphically) in the study report.
	Metric 25:	Reporting of Data	Medium	× 2	4	The study report showed data by exposure group in most cases (i.e., for strain TA 100). It was presumed that trichloroacetyl chloride was also tested in TA 98 and the results were negative, but this is not expicitly stated. This omission does not impact the study results for strain TA 100.
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 52: **In vitro evaluation results for Vamvakas et al 1987 for S. typhimurium mutagenicity study**

Study Citation:	S. Vamvakas, W. Dekant, K. Berthold, S. Schmidt, D. Wild, D. Henschler (1987). Enzymatic transformation of mercapturic acids derived from halogenated alkenes to reactive and mutagenic intermediates Biochemical Pharmacology, 36(17,17), 2741-2748
Data Type:	Preincubation assay - PERC
HERO ID:	65133

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name as PERC metabolite, S-trichlorovinyl-N-acetylcysteine (N-Ac-TCVC), CASRN was not reported.
	Metric 2:	Test Substance Source	High	× 1	1	The compound was synthesized (methods provided), and analytically verified.
	Metric 3:	Test Substance Purity	High	× 1	1	Purity >99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	Use of a concurrent negative control was not reported, nor were control results reported graphically.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Use of a concurrent positive control was not used or reported, but the results were reported to be positive.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were performed as described in another study with minimal additional details.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study design
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation of test solutions and storage were not reported.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Exposure methods were cited to another publication with no additional details
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations tested were not reported, but could be determined from data shown graphically
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration (120 min) was reported and appropriate for the study type.
	Metric 12:	Exposure Route and Method	High	× 1	1	3-4 exposure groups were tested for each assay condition. A dose-response was observed so the concentrations and spacing were appropriate for the outcome of interest.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Metabolic activation was reported (male Wistar rat kidney supernatant), and the concentration added was reported. Additional details on the source , isolation and other methodological details were not provided.
Domain 4: Test Model						

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Study Citation:	S. Vamvakas, W. Dekant, K. Berthold, S. Schmidt, D. Wild, D. Henschler (1987). Enzymatic transformation of mercapturic acids derived from halogenated alkenes to reactive and mutagenic intermediates Biochemical Pharmacology, 36(17,17), 2741-2748
Data Type:	Preincubation assay - PERC
HERO ID:	65133

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 14: Test Model	Low	× 2	6	S. typhimurium strain TA100 was reported. No additional details (including source) were reported.
	Metric 15: Number per Group	Medium	× 1	2	Only a single strain was tested which is lower than the typical number used for this study type. The assays were performed in triplicate.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	Not Rated	NA	NA	Outcome assessment methodology was not described (assay cited to another publication).
	Metric 17: Consistency of Outcome Assessment	Not Rated	NA	NA	Outcome assessment was not described (assay cited to another publication).
	Metric 18: Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study type
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study type
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial batch/lot number of organisms used per group was not reported.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	Not Rated	NA	NA	Statistical methods were not used. Even though studies were performed in triplicate, measures of variance were not provided.
	Metric 23: Data Interpretation	Low	× 2	6	Scoring and evaluation criteria were not explicitly reported but text mentions doubling of spontaneous revertants which appears to be criterion for a positive result. Source of the number of spontaneous revertants was not reported but does not appear to be concurrent control.
	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	Medium	× 2	4	Data were reported graphically for the all treatment groups (means only; no measure of variability)
Overall Quality Determination‡		Unacceptable⋆⋆		2.2	
Extracted		No			

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Study Citation:	S. Vamvakas, W. Dekant, K. Berthold, S. Schmidt, D. Wild, D. Henschler (1987). Enzymatic transformation of mercapturic acids derived from halogenated alkenes to reactive and mutagenic intermediates Biochemical Pharmacology, 36(17,17), 2741-2748
Data Type:	Preincubation assay - PERC
HERO ID:	65133

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 =≥ 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 53: **In vitro evaluation results for Connor et al 1985 for bacterial mutagenicity study**

Study Citation:	T. H. Connor, J. C. Theiss, H. A. Hanna, D. K. Monteith, T. S. Matney (1985). Genotoxicity of organic chemicals frequently found in the air of mobile homes Toxicology Letters, 25(1,1), 33-40
Data Type:	Mutagenic bacterial assay
HERO ID:	74926

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified as tetrachloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	The source of test substance was identified as Eastman Kodak.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity and/or grade of test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Controls were indicated by a footnote to Table 1; however, details regarding the negative control group were not reported.
	Metric 5:	Positive Controls	Medium	× 2	4	Positive controls were run and yeilded positive results, however it is not stated if these tests were run concurrently with experiment.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and/or cited in another publication (Maron and Ames, 1983), but appeared to be appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Preparation was described. Test substance was prepared immediately prior to use.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure was consistent across the treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration stated to be 48 hrs.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure and dose spacing were reported and appropriate.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Method of preparing liver homogenate (S9) from Aroclor-induced male Sprague-Dawley rat liver was not reported.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was reported but no additional details were reported. The source of Salmonella typhimurium strains TA100 and TA98 was not identified. UTH strains are not commonly used.

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Study Citation:	T. H. Connor, J. C. Theiss, H. A. Hanna, D. K. Monteith, T. S. Matney (1985). Genotoxicity of organic chemicals frequently found in the air of mobile homes Toxicology Letters, 25(1,1), 33-40
Data Type:	Mutagenic bacterial assay
HERO ID:	74926

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 15: Number per Group	Medium	× 1	2	Assay was performed in duplicate.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18: Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable.
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	Blinding was not necessary.
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions were not reported for each across treatment group.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	Not Rated	NA	NA	Quantitative data were not provided.
	Metric 23: Data Interpretation	High	× 2	2	Scoring criteria was consistent with standards.
	Metric 24: Cytotoxicity Data	Low	× 1	3	Toxicity was used to determine the upper limit of the dose tested; however, the method for evaluating cytotoxicity was not described.
	Metric 25: Reporting of Data	Low	× 2	6	Data for exposure-related findings were not shown for each study group (indicated as negative for all doses in text).
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 54: **Animal toxicity evaluation results of Beliles et al 1980 for a 3-wk gestational inhalation study on genotoxicity in vivo (mechanistic) outcomes**

Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	in vivo genotoxicity
HERO ID:	58331

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name and synonym
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer and lot number given.
	Metric 3:	Test Substance Purity	Medium	× 1	2	91% pure, impurities were not characterized
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Filtered air controls; "To avoid exposure of control animals to test materials, all control chambers were in a different chamber room than the exposure chambers. No test materials were taken into the control rooms."
	Metric 5:	Positive Controls	High	× 1	1	Positive controls (reference mutagens) were used for all studies. "However, the contractor did not attempt to verify the purity of these commercially available samples."
	Metric 6:	Randomized Allocation	High	× 1	1	"The animals were randomly assigned to experimental groups."
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Method and equipment used to generate the test substance as a vapor were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and analytical concentrations were provided. Range of measure concentration did not deviate more than 10% target concentration.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	The exposure frequency and duration were reported and appropriate for this study.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	2 exposure concentrations (100 and 500ppm)
	Metric 12:	Exposure Route and Method	High	× 1	1	Dynamic chamber , whole body, assumed that chemical does not condense.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain and source were reported; starting age and body weight not given.

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Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	in vivo genotoxicity
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	well reported
	Metric 15:	Number per Group	High	× 1	1	6-10/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Dominant lethal assay, spermhead abnormality, chromosomal aberration in rat bone marrow, rat dominant lethal test conducted.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	High	× 1	1	
	Metric 19:	Blinding of Assessors	Medium	× 1	2	Blinding was not reported, but most outcomes were not subjective.
	Metric 20:	Negative Control Response	High	× 1	1	
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	High	× 2	2	None related to genotoxicity
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	None related to genotoxicity
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistics were well described and appropriate
	Metric 24:	Reporting of Data	High	× 2	2	All outcomes were reported.
Overall Quality Determination‡			High		1.2	
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

Table 55: **In vitro evaluation results for Costa and Ivanetich 1984 for rat hepatocyte unscheduled DNA synthesis study**

Study Citation:	A. K. Costa, K. M. Ivanetich (1984). Chlorinated ethylenes: their metabolism and effect on DNA repair in rat hepatocytes Carcinogenesis, 5(12,12), 1629-1636
Data Type:	UDS for perc
HERO ID:	75075

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as perchloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (a manufacturer). Although a batch/lot number were not reported, the test substance is not expected to vary in composition.
	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	Medium	× 2	4	The study authors report using a concurrent negative controls. DMSO was used as negative control substance (data shown); vehicle-only (ethanol) controls were also used (data not shown).
	Metric 5:	Positive Controls	High	× 2	2	Benzo[a]pyrene, a known carcinogen, was used as a positive control, and the intended positive response was induced.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were partially described and cited to Andrae and Schwarz (1981). Equipment used to measure absorbance was not reported.
	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	Test substance preparation was reported (dissolved in ethanol); storage was not reported (but was unlikely to affect the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposures were reported to be administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The test concentration was reported without ambiguity (2.5 mM).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (2.5 hours) and considered appropriate for the study type (i.e., effective based on positive findings).

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Study Citation:	A. K. Costa, K. M. Ivanetich (1984). Chlorinated ethylenes: their metabolism and effect on DNA repair in rat hepatocytes Carcinogenesis, 5(12,12), 1629-1636
Data Type:	UDS for perc
HERO ID:	75075

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	One concentration was used. This dose was justified by the study authors as "the highest concentration..tolerated by the hepatocytes." Although results were negative, it is presumed that the test substance was tested at the highest possible concentration without excessive cytotoxicity.
	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 and is routinely used for the outcome of interest. The source of parent animals was not reported.
	Metric 15:	Number per Group	Medium	× 1	2	Experiments were reportedly repeated in as second set of experiments.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology appeared 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	This method is not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This method is not applicable to the study type.
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. The study authors indicated that each experiment was conducted using hepatocytes from a single rat; viability of hepatocytes (>90%) was verified prior to use.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analysis is not required by study type (statistics were performed in the study, but not for this assay). Results (expressed in dpm and absorbance at 260 nm) were shown graphically.
	Metric 23:	Data Interpretation	Low	× 2	6	The study indicated that UDS was identified by a radioactive peak binding with parental DNA (coincident with the absorbance peak at 260 nm). Based on the data shown graphically, the determination/threshold for a positive result appears to be somewhat subjective.

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Study Citation:	A. K. Costa, K. M. Ivanetich (1984). Chlorinated ethylenes: their metabolism and effect on DNA repair in rat hepatocytes Carcinogenesis, 5(12,12), 1629-1636
Data Type:	UDS for perc
HERO ID:	75075

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	The study indicated that the viability of cells was evaluated using the Trypan blue exclusion assay (without additional details). no data were shown.
	Metric 25:	Reporting of Data	Low	× 2	6	Data for the outcome was presented for the control and treatment group for one set of hepatocytes from a phenobarbital treated rat; a second set of experiments was noted to have identical results (+/- 5%, but was not reported). Data for the ethanol vehicle control were not shown, but reported to not stimulate UDS.
Overall Quality Determination‡			High		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 56: **In vitro evaluation results of Watanabe et al 1998 for a study on bacterial reverse mutation**

Study Citation:	K. Watanabe, K. Satamoto, T. Sasaki (1998). Comparisons on chemically-induced mutation among four bacterial strains, Salmonella typhimurium TA102 and TA2638, and Escherichia coli WP2/pKM101 and WP2 uvrA/pKM101: Collaborative study II Mutation Research, 412(1,1), 17-31
Data Type:	Bacterial reverse mutation for Perc
HERO ID:	194631

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. A CASRN was also provided.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance (a manufacturer) was reported. Although a batch/lot number was not provided, it was indicated that the same lot of each chemical was used for all experiments.
	Metric 3:	Test Substance Purity	Medium	× 1	2	The study did not indicate the purity of the test substance; however, it was indicated that chemicals used in the study were of the 'highest purity.' It is expected that observed effects are due to the test substance itself; the omission of the specific purity of the test substance is not likely to impact the study results.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study used negative controls; all conditions except exposure appeared to be equal. It was not explicitly specified (but it was inferred from the study) that the negative control was a solvent-only (DMSO-only) control.
	Metric 5:	Positive Controls	Medium	× 2	4	A concurrent positive control was reportedly used (2-aminoanthrecene in the presence of activation). Although the study noted that increased numbers of revertant colonies were observed in all strains with the positive controls in all experiments, positive control data were not shown. This omission is unlikely to have a substantial impact on results.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were briefly described, and partially cited to another publication (Watanabe et al. 1996).
	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 inferred from the test (i.e., dissolved in DMSO), but storage was not reported (unlikely to affect results owing to the short duration of the study).

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Study Citation:	K. Watanabe, K. Satamoto, T. Sasaki (1998). Comparisons on chemically-induced mutation among four bacterial strains, Salmonella typhimurium TA102 and TA2638, and Escherichia coli WP2/pKM101 and WP2 uvrA/pKM101: Collaborative study II Mutation Research, 412(1,1), 17-31
Data Type:	Bacterial reverse mutation for Perc
HERO ID:	194631

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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	Doses were reported without ambiguity (Appendix A).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The duration of the study was reported and consistent with other studies of this type.
	Metric 12:	Exposure Route and Method		× 1	NA	The study used 6 doses plus controls (5 analyzable doses in most strains owing to toxicity). The doses selected appeared appropriate to evaluate dose-response and the test was conducted up to a dose that caused cytotoxicity.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The study authors reported that exposures were conducted in the presence of metabolic activation; the source and concentration in final culture were described. The type (rat, mouse, hamster) of S9 was not reported, but this is unlikely to impact the study results.
Domain 4: Test Model						
	Metric 14:	Test Model	Not Rated	NA	NA	The study indicated that details associated with the bacterial strains were described in another publication (Watanabe et al. 1996). The characteristic properties of bacterial strains used were reported in the introduction of the study.
	Metric 15:	Number per Group	High	× 1	1	The study indicated that there were three plates per dose. In addition, it was noted that the test chemical was subjected to at least two independent experiments in two laboratories.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology (counting of revertant colonies after 48 hours incubation) addressed or reported the intended outcome of interest (mutagenicity).
	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		× 1	NA	This metric is not applicable to the study type.
Domain 6: Confounding / Variable Control						

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Study Citation:	K. Watanabe, K. Satamoto, T. Sasaki (1998). Comparisons on chemically-induced mutation among four bacterial strains, Salmonella typhimurium TA102 and TA2638, and Escherichia coli WP2/pKM101 and WP2 uvrA/pKM101: Collaborative study II Mutation Research, 412(1,1), 17-31
Data Type:	Bacterial reverse mutation for Perc
HERO ID:	194631

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	The study explicitly specified that precautions were taken to ensure that there were no differences among the initial study parameters (the bacterial strains used from a central source, the same lot of test substance used in all experiments).
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported (not likely to substantially impact the study results).
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	The study indicates that data were analyzed using a linear regression test (based on a recommendation for this type of analysis from a cited publication) and using a significance level of 1%. Data provided in the study were not amenable to independent analysis (mean with no measure of variance provided).
	Metric 23:	Data Interpretation	Medium	× 2	4	The study indicated that the statistical analysis used was based on the dose-response relationship. Therefore, it is inferred from the text that the dose-relatedness/statistical significance of the response was the criteria for a positive response.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were defined (as a reduction in the background lawn and/or a reduction in the number of revertant colonies), but the methods of measurements were not fully described or reported.
	Metric 25:	Reporting of Data	High	× 2	2	Results were reported 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 57: **In vitro evaluation results of Doherty et al 1996 study on a micronucleus assay**

Study Citation:	A. T. Doherty, S. Ellard, E. M. Parry, J. M. Parry (1996). An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells Mutagenesis, 11(3,3), 247-274
Data Type:	Micronucleus assay for perc
HERO ID:	194804

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance is clearly identified by name (tetrachloroethylene).
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported. The test substance was obtained from a manufacturer. Although a batch/lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity/grade of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The report indicates that the study authors used concurrent negative control groups. It appears that all conditions were equal except exposure to the test substance.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Although a concurrent positive control group was not used, the response for chemicals used in the study was positive and exposure-related. Therefore, a positive control is not absolutely required.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures (including cell density, culture media, incubation temperatures, washing/rinsing methods, and slide preparation) were decribed. Details of some procedures (e.g., kinetochore labeling) were cited to other publications. Although procedures deviated somewhat from customary practices, they appeared to be applicable to 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	High	× 1	1	Preparation conditions were reported. It was indicated that, owing to insolubility of the test substances (in general), stock solutions were prepared in growth medium at the top concentration to be tested and were placed in an incubator (with shaking) overnight, and then diluted.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration appeared to be consistent across study groups.

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Study Citation:	A. T. Doherty, S. Ellard, E. M. Parry, J. M. Parry (1996). An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells Mutagenesis, 11(3,3), 247-274
Data Type:	Micronucleus assay for perc
HERO ID:	194804

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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 exposure duration was reported and appropriate for the study type. It was noted that, owing to the protocol being used (i.e., use of genetically modified cell lines rather than S9), the exposure duration could be extended to encompass the whole cell cycle (18 hours for AHH-1 cells and 24 hours for MCL-5 and h2E1 cell lines).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (5 plus controls) and concentration spacing were considered adequate to address the purpose of the study (e.g., evaluation of exposure-response relationships). Concentrations up to 5 mM were used.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	The study was conducted using metabolically competent cells (rather than an exogenous activation system). The parental cell line used in the study (AHH-1) had only a low level of native CYP1A1 activity; the other two cell lines enabled activation via additional CYP enzymes (CYP2E1 for h2E1 cells, and CYP2E1, 1A2, 2A6, 3A4 and epoxide hydrolase). The study states that genetically modified cells lines such as those used in this study have been shown in other studies to detect metabolites produced from indirect-acting compounds.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The cell lines used in the study were obtained from a commericial source (Gentest Corporation); information was provided as to how the MCL-5 and h2E1 strains were derived from the parent (AHH-1 cell line). It was noted as well that the cell lines were cultures for up to 5 weeks to maintain a stable karyotype. The study states that genetically engineered human lymphoblastoid cell lines have been used previously to evaluate clastogenic and aneugenic substances.
	Metric 15:	Number per Group	High	× 1	1	Duplicate cultures were utilized. The number of replicates was reported and was appopriate for the study type.
Domain 5: Outcome Assessment						

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Study Citation:	A. T. Doherty, S. Ellard, E. M. Parry, J. M. Parry (1996). An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells Mutagenesis, 11(3,3), 247-274
Data Type:	Micronucleus assay for perc
HERO ID:	194804

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the outcome of interest and appeared to be sensitive to the outcome of interest. In addition to evaluating micronucleus formation, the study went on to characterize the response (via kinetochore labeling to differentiate between aneugenic and clastogenic mechanisms).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessments were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The study reported adequate sampling for the outcome of interest. It was indicated that 1000 binucleate cells per culture (2000 per exposure level) were examined for the presence of micronuclei (standard for studies of this 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	No confounding differences in test design/procedures among study groups were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding differences with respect to outcomes unrelated to exposure were identified.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	The study indicates that significant effects (with respect to micronuclei induction) reported in the results and discussion were based on significance in the X2 test at the 99% confidence limit. The results section describes statistically significantly increased micronuclei formation in the various cell lines, largely without reference to specific exposure levels. The accompanying table (Table 1) and figures do not provide indications of statistical significance. The "lowest significant dose" of induction of kinetchore positive/negative nuclei (from replicate experiments) was provided in an additional table (Table 2). Omissions in reporting the application of statistical methods is not expected to substantially impact the study results.
	Metric 23:	Data Interpretation	Medium	× 2	4	The study authors eluded to (but did not expicitly report) the evaluation criteria (i.e., a statistically significantly increase in micronuclei); the evaluation criteria are consistent with studies of this type.

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Study Citation:	A. T. Doherty, S. Ellard, E. M. Parry, J. M. Parry (1996). An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells Mutagenesis, 11(3,3), 247-274
Data Type:	Micronucleus assay for perc
HERO ID:	194804

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Cytotoxicity Data	Medium	× 1	2	The study indicates that relative toxicity was evaluated as the proportion of binucleate and mononucleate cells; the proportion of binucleate cells provides an estimate of the nuclear cell division index and this a measure of toxicity. Although the assessment of cytotoxicity was not fully described/accounted for, these omissions are not likely to substantially impact the study results.
	Metric 25: Reporting of Data	High	× 2	2	Data for exposure-related outcomes were reported 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 58: **In vitro evaluation results of Doherty et al 1996 for micronucleus assay**

Study Citation:	A. T. Doherty, S. Ellard, E. M. Parry, J. M. Parry (1996). An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells Mutagenesis, 11(3,3), 247-274
Data Type:	Micronucleus assay_CCl4
HERO ID:	194804

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance is clearly identified by name (carbon tetrachloride).
	Metric 2:	Test Substance Source	Low	× 1	3	The test substance was not obtained from a manufacturer, but was supplied as a gift (from Dr. R. Crebelli in Rome). Although there did not appear to be analytical verification of the test substance in this study, this study cited publications by Dr. Crebelli (including studies of chlorinated hydrocarbons).
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity/grade of the test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The report indicates that the study authors used concurrent negative control groups (vehicle was indicated to be culture medium). It appears that all conditions were equal except exposure to the test substance.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Although a concurrent positive control group was not used, the response for CCl4 (and other chemicals) was positive and exposure-related. Therefore, a positive control is not absolutely required.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Methods and procedures (including cell density, culture media, incubation temperatures, washing/rinsing methods, and slide preparation) were decribed. Details of some procedures (e.g., kinetochore labeling) were cited to other publications. Although procedures deviated somewhat from customary practices, they appeared to be applicable to 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						

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Study Citation:	A. T. Doherty, S. Ellard, E. M. Parry, J. M. Parry (1996). An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells Mutagenesis, 11(3,3), 247-274
Data Type:	Micronucleus assay_CCl4
HERO ID:	194804

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation conditions were reported. It was indicated that, owing to insolubility of the test substances (in general), stock solutions were prepared in growth medium at the top concentration to be tested and were placed in an incubator (with shaking) overnight, and then diluted. It was not specified what methods were conducted to minimize loss of the volatile test substance, but it was noted that the exposures were carried out in glass vials, which were assumed to be closed systems for the duration of the exposure; therefore, this is not considered to have substantially impacted the results.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration appeared to be consistent 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 exposure duration was reported and appropriate for the study type. It was noted that, owing to the protocol being used (i.e., use of genetically modified cell lines rather than S9), the exposure duration could be extended to encompass the whole cell cycle (18 hours for AHH-1 cells and 24 hours for MCL-5 and h2E1 cell lines).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (4 plus control) and concentration spacing were considered adequate to address the purpose of the study (e.g., evaluation of exposure-response relationships). Concentrations up to 10 mM were used, which is standard for studies of this type.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The study was conducted using metabolically competent cells (rather than an exogenous activation system). The parental cell line used in the study (AHH-1) had only a low level of native CYP1A1 activity; the other two cell lines enabled activation via additional CYP enzymes (CYP2E1 for h2E1 cells, and CYP2E1, 1A2, 2A6, 3A4 and epoxide hydrolase). The study states that genetically modified cells lines such as those used in this study have been shown in other studies to detect metabolites produced from indirect-acting compounds.
Domain 4: Test Model						

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Study Citation:	A. T. Doherty, S. Ellard, E. M. Parry, J. M. Parry (1996). An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells Mutagenesis, 11(3,3), 247-274
Data Type:	Micronucleus assay_CCl4
HERO ID:	194804

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The cell lines used in the study were obtained from a commericial source (Gentest Corporation); information was provided as to how the MCL-5 and h2E1 strains were derived from the parent (AHH-1 cell line). It was noted as well that the cell lines were cultures for up to 5 weeks to maintain a stable karyotype. The study states that genetically engineered human lymphoblastoid cell lines have been used previously to evaluate clastogenic and aneugenic substances.
	Metric 15:	Number per Group	High	× 1	1	Duplicate cultures were utilized. The number of replicates was reported and was appopriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology addressed the outcome of interest and appeared to be sensitive to the outcome of interest. In addition to evaluating micronucleus formation, the study went on to characterize the response (via kinetochore labeling to differentiate between aneugenic and clastogenic mechanisms).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessments were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The study reported adequate sampling for the outcome of interest. It was indicated that 1000 binucleate cells per culture (2000 per exposure level) were examined for the presence of micronuclei (standard for studies of this type).
	Metric 19:	Blinding of Assessors	High	× 1	1	It was reported that slides were coded prior to analysis.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding differences in test design/procedures among study groups were identified.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	High	× 1	1	No confounding differences with respect to outcomes unrelated to exposure were identified.
Domain 7: Data Presentation and Analysis						

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Study Citation:	A. T. Doherty, S. Ellard, E. M. Parry, J. M. Parry (1996). An investigation into the activation and deactivation of chlorinated hydrocarbons to genotoxins in metabolically competent human cells Mutagenesis, 11(3,3), 247-274
Data Type:	Micronucleus assay_CCl4
HERO ID:	194804

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	High	× 1	1	The study indicates that significant effects (with respect to micronuclei induction) reported in the results and discussion were based on significance in the Chi-squared test at the 99% confidence limit. The results section describes statistically significantly increased micronuclei formation in the various cell lines, largely without reference to specific exposure levels. The accompanying table (Table I-ix for CCl4) and figures do not provide indications of statistical significance; however, raw data are provided, enabling independent statistical analysis. The "lowest significant dose" of induction of kinetochore positive/negative nuclei (from replicate experiments) was provided in an additional table (Table II).
	Metric 23:	Data Interpretation	Medium	× 2	4	The study authors alluded to (but did not explicitly report) the evaluation criteria (i.e., a statistically significantly increase in micronuclei); the evaluation criteria are consistent with studies of this type.
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	The study indicates that relative toxicity was evaluated as the proportion of binucleate and mononucleate cells; the proportion of binucleate cells provides an estimate of the nuclear cell division index and this a measure of toxicity. Although the assessment of cytotoxicity was not fully described/accounted for, these omissions are not likely to substantially impact the study results. For example, toxicity at 10 mM CCl4 in all cell lines appeared to be >55% relative to the negative control; however, micronuclei formation was seen at lower exposure concentrations in the absence of substantial (relative) toxicity.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related outcomes 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 59: **In vitro evaluation results of Roldán-Arjona et al 1991 study on ara mutagenicity assay in S. typhimurium**

Study Citation:	T. Roldán-Arjona, M. D. García-Pedrajas, F. L. Luque-Romero, C. Hera, C. Pueyo (1991). An association between mutagenicity of the ara test of salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons Mutagenesis, 6(3,3), 199-205
Data Type:	ara mutagenicity assay in S. typhimurium- Perc
HERO ID:	194881

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as Tetrachloroethylene ("TTCEL") with the correct CASRN and molecular formula.
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (Aldrich). The product number and batch/lot number were not reported, but substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity and/or grade of the test substance was reported (provided by the supplier). 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors report using a solvent control (DMSO)
	Metric 5:	Positive Controls	Not Rated	NA	NA	A concurrent positive control was not used but may not be required for this study. The response of some known carcinogens tested in the study were positive and exhibited a dose-related response for mutations; this indicates that the assay was effective at inducing and identifying a positive mutagenic response.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were described; more detailed assay procedures were also described in a previously published studies (Hera and Pueyo, 1986; Roldan-Arjona et al., 1989)
	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	High	× 1	1	Test substance preparation was described (dissolved in DMSO). Test substance storage was not reported, but this is appropriate given the study design (single-dose administration).
	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 Table III without ambiguity

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Study Citation:	T. Roldán-Arjona, M. D. García-Pedrajas, F. L. Luque-Romero, C. Hera, C. Pueyo (1991). An association between mutagenicity of the ara test of salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons Mutagenesis, 6(3,3), 199-205
Data Type:	ara mutagenicity assay in S. typhimurium- Perc
HERO ID:	194881

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (20 minutes) and considered appropriate, as it yielded positive responses from a variety of chemicals tested and was in line with the Ames bacterial reverse mutation assay preincubation method exposure duration (also 20 minutes according to current standards).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number and spacing of exposure concentrations were reported in the results. It was noted that the investigator used a wide range of doses and the compound (negative for mutagenicity) gave a lethal response which indicated that bacteria were adequately exposed
	Metric 13:	Metabolic Activation	Medium	× 1	2	Assays were conducted with and without metabolic activation (S9 fraction from male rat liver induced with Aroclor-1254). The preparation of the S9 fraction was described in a previous publication (Maron and Ames, 1983). The source, concentration in the final culture and quality control information were not reported.
Domain 4: Test Model						
	Metric 14:	Test Model	Not Rated	NA	NA	The test model was reported along with limited descriptive information. The test model was routinely used for the outcome of interest. (S. typhimurium strains BA13 and BAL 13). The source of the bacteria strains were not specified in the report. These strains have been previously described in previously published reports (Ruiz-Rubio et al., 1985; Roldan-Arjona et al„ 1989)
	Metric 15:	Number per Group	Low	× 1	3	It was reported that at least two plates per dose level were used. This is not considered adequate by current standards for a similar assay (Ames bacterial reverse mutation requires 3 plates per dose level; use of 2 plates per dose level must be scientifically justified). Furthermore, the uncertainty regarding the number of plates per dose level ("at least two") indicates that the data yielded from each test substance and dose level were not obtained by identical procedures.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The AraR bacterial forward mutation assay appeared to be appropriate for the outcome of interest.

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Study Citation:	T. Roldán-Arjona, M. D. García-Pedrajas, F. L. Luque-Romero, C. Hera, C. Pueyo (1991). An association between mutagenicity of the ara test of salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons Mutagenesis, 6(3,3), 199-205
Data Type:	ara mutagenicity assay in S. typhimurium- Perc
HERO ID:	194881

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	The use of "at least two" plates per dose level indicates that the data yielded from each test substance and dose level were not obtained by identical procedures. It is not clear what the maximum amount of plates per dose level was, so the range of replicates used per dose level is unknown. This is considered to have potentially impacted results.
	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	High	× 1	1	No confounding variable unrelated to exposure were identified
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	A calculation for correlating number of mutations per unit time and per unit dose ("mutagenic potency") with previously established carcinogenic potency was given. However, statistical analysis was not conducted on the data. Although means and standard deviations are provided for each dose level, the number of plates per dose level is uncertain, and therefore independent statistical analysis cannot be conducted. However, statistical analysis is not necessarily required for the Ames bacterial reverse mutation assay, and due to the similarity of the AraR bacterial forward mutation assay, statistical analysis is considered to be not necessarily required for the present data.
	Metric 23:	Data Interpretation	High	× 2	2	The evaluation criteria were reported and appropriate (test compound was considered mutagenic of the number of AraR mutant colonies was at least twice the value of the corresponding solvent control, over at least three dose levels)
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity endpoints were described (survival)
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcome was presented for the control and treatment groups
Overall Quality Determination‡			High		1.3	

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Study Citation:	T. Roldán-Arjona, M. D. García-Pedrajas, F. L. Luque-Romero, C. Hera, C. Pueyo (1991). An association between mutagenicity of the ara test of salmonella typhimurium and carcinogenicity in rodents for 16 halogenated aliphatic hydrocarbons Mutagenesis, 6(3,3), 199-205
Data Type:	ara mutagenicity assay in S. typhimurium- Perc
HERO ID:	194881

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 60: **In vitro evaluation results for Milman et al 1988 for bacterial reverse mutation study**

Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	Perc bacterial reverse mutation
HERO ID:	200479

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.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was reported as a range for multiple compounds (97-99% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	A concurrent negative control group was not included or reported.
	Metric 5:	Positive Controls	Unacceptable	× 2	8	A concurrent positive control or proficiency group was not used. A positive control is very commonly utilized in a bacterial reverse mutation assay. However, some test substances yielded positive responses, demonstrating that the assay was able to detect a positive response.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay procedures were cited to other publications (Ames et al., 1973a,b, 1975).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage was not reported.
	Metric 9:	Consistency of Exposure Administration	Unacceptable	× 1	4	Critical exposure details (e.g., amount of test substance used) were not reported.
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	The exposure doses/concentrations or amounts of test substance were not reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	No information on exposure duration(s) was reported, although assay procedure details were cited to other references.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups and dose/concentration spacing were not reported.
	Metric 13:	Metabolic Activation	Medium	× 1	2	A commonly used metabolic activation system was reported in the study; however, some details regarding type, composition mix, concentration, or quality control information were not described
Domain 4: Test Model						

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Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	Perc bacterial reverse mutation
HERO ID:	200479

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 14: Test Model	Low	× 2	6	The test model was reported but no additional details were given.
	Metric 15: Number per Group	Unacceptable	× 1	4	Replicates per study group were not reported.
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported and sensitive for the outcome of interest.
	Metric 17: Consistency of Outcome Assessment	Low	× 1	3	Details were not reported regarding the execution of the study protocol for outcome assessment.
	Metric 18: Sampling Adequacy	Not Rated	NA	NA	Not applicable to the outcome of interest.
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	Not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control					
	Metric 20: Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions per study group were not reported.
	Metric 21: Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	Unacceptable	× 1	4	No quantitative data were provided.
	Metric 23: Data Interpretation	Medium	× 2	4	Evaluation criteria were partially 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	Low	× 2	6	It was reported in the text that "no reproducible, dose-related increase in the number of [...] revertants" was observed for Perc. No quantitative data was reported.
Overall Quality Determination‡		Unacceptable⋆⋆		3.5	
Extracted		No			

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Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	Perc bacterial reverse mutation
HERO ID:	200479

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 61: **In vitro evaluation results for Milman et al 1988 for hepatocyte DNA repair study**

Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	Perc hepatocyte DNA repair
HERO ID:	200479

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.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was reported as a range for multiple compounds (97-99% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Unacceptable	× 2	8	A concurrent negative control group was not included or reported.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric may not be applicable to the DNA repair test.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay procedures were cited to other publications (Williams 1976, 1977).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage was not reported.
	Metric 9:	Consistency of Exposure Administration	Unacceptable	× 1	4	Critical exposure details (e.g., amount of test substance used) were not reported.
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	The exposure doses/concentrations or amounts of test substance were not reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	No information on exposure duration(s) was reported, although assay procedure details were cited to other references.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups and dose/concentration spacing were not reported.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not needed for primary hepatocytes.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was reported but no additional details were given.
	Metric 15:	Number per Group	High	× 1	1	Triplicates were indicated.
Domain 5: Outcome Assessment						

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Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	Perc hepatocyte DNA repair
HERO ID:	200479

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Details were not reported regarding the execution of the study protocol for outcome assessment.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions per study group were not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	No quantitative data were provided.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were partially 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	Low	× 2	6	Text indicated that Perc was negative in both rats and mice. No quantitative data was provided.
Overall Quality Determination‡			Unacceptable⋆⋆		3.0	
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 =≥ 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 62: **In vitro evaluation results for Milman et al 1988 for cell transformation study**

Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	Perc cell transformation
HERO ID:	200479

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.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was reported.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Purity was reported as a range for multiple compounds (97-99% pure).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	A negative control was referenced briefly in the results, but no details were provided and results were not reported for negative controls.
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric may not be applicable to the cell transformation assay.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay procedures were cited to other publications (Sivak and Tu, 1980).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Information on preparation and storage was not reported.
	Metric 9:	Consistency of Exposure Administration	Unacceptable	× 1	4	Critical exposure details (e.g., amount of test substance used) were not reported.
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	The exposure doses/concentrations or amounts of test substance were not reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Not Rated	NA	NA	No information on exposure duration(s) was reported, although assay procedure details were cited to other references.
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	The number of exposure groups and dose/concentration spacing were not reported.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not needed.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was reported but no additional details were given.
	Metric 15:	Number per Group	Not Rated	NA	NA	Not indicated; possibly cited to another publication (Sivak and Tu, 1980)
Domain 5: Outcome Assessment						

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Study Citation:	H. A. Milman, D. L. Story, E. S. Riccio, A. Sivak, A. S. Tu, G. M. Williams, C. Tong, C. A. Tyson (1988). Rat liver foci and in vitro assays to detect initiating and promoting effects of chlorinated ethanes and ethylenes Annals of the New York Academy of Sciences, 534 521-530
Data Type:	Perc cell transformation
HERO ID:	200479

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported and sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Details were not reported regarding the execution of the study protocol for outcome assessment.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial conditions per study group were not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on outcome differences unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	No quantitative data were provided.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were partially reported.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity was assessed; however, methods were not described.
	Metric 25:	Reporting of Data	Low	× 2	6	Text indicated that Perc was negative. No other details were provided.
Overall Quality Determination‡			Unacceptable⋆⋆		2.8	
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 =≥ 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 63: **In vitro evaluation results for Sofuni et al 1985 for chromosomal aberration study**

Study Citation:	T. Sofuni, M. Hayashi, A. Matsuoka, M. Sawada, M. Hatanaka,Ishidate M Jr (1985). [Mutagenicity tests on organic chemical contaminants in city water and related compounds. II. Chromosome aberration tests in cultured mammalian cells] Kokuritsu Iyakuhin Shokuhin Eisei Kenkyu¯jo Ho¯koku / Bulletin of the National Institute of Health Sciences, 103(103,103), 64-75
Data Type:	Chromosomal aberrations_Perc
HERO ID:	201741

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 (tetrachloroethylene).
	Metric 2:	Test Substance Source	Not Rated	NA	NA	Study details are not available because it is a foreign language study; only the abstract and data tables are provided in English.
	Metric 3:	Test Substance Purity	Not Rated	NA	NA	Study details are not available because it is a foreign language study; only the abstract and data tables are provided in English.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	It is inferred from the data tables that concurrent negative control groups were used (DMSO-only controls).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Although a concurrent positive control group was not used, the response for some of the chemicals in the study was positive and exposure-related. Therefore, a positive control is not absolutely required.
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Study details are not available because it is a foreign language study; only the abstract and data tables are provided in English.
	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	Study details are not available because it is a foreign language study; only the abstract and data tables are provided in English.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Study details are not available because it is a foreign language study; only the abstract and data tables are provided in English.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations were reported without ambiguity.

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Study Citation: T. Sofuni, M. Hayashi, A. Matsuoka, M. Sawada, M. Hatanaka,Ishidate M Jr (1985). [Mutagenicity tests on organic chemical contaminants in city water and related compounds. II. Chromosome aberration tests in cultured mammalian cells] Kokuritsu Iyakuhin Shokuhin Eisei Kenkyu¯jo Ho¯koku / Bulletin of the National Institute of Health Sciences, 103(103,103), 64-75

Data Type: Chromosomal aberrations_Perc

HERO ID: 201741

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The duration of exposure was reported (24 and 48 hours) for experiments performed in the absence of activation (Table 1) and was considered appropriate for the study type. The duration of exposure for studies conducted with a metabolic activation system was not provided in Table 4 (tables and abstract only provided in English).
	Metric 12:	Exposure Route and Method	High	× 1	1	At least three analyzable concentrations of the test substance were used in the presence/absence of activation. Although results were negative, it was clear that the doses tested were high enough, as the highest dose produced cytotoxicity (not analyzable).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	The use of a metabolic activation system was reported. Details with respect to the source/preparation of the activation system were not available; only the abstract and data tables are provided in English.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test system used (Chinese hamster lung cells) in routinely used and is considered appropriate for the study type (evaluation of chromosomal aberrations).
	Metric 15:	Number per Group	Not Rated	NA	NA	It is not clear from the information provided (abstract and tables only were provided in English) if single or multiple cultures were used.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment addressed the intended outcome of interest (chromosomal aberrations). Numbers of chromatid gaps, breaks, and exchanges and chromosome breaks and exchanges were assessed.
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Study details are not available because it is a foreign language study; only the abstract and data tables are provided in English.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Study details are not available because it is a foreign language study; only the abstract and data tables are provided in English.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Study details are not available because it is a foreign language study; only the abstract and data tables are provided in English.
Domain 6: Confounding / Variable Control						

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Study Citation:	T. Sofuni, M. Hayashi, A. Matsuoka, M. Sawada, M. Hatanaka,Ishidate M Jr (1985). [Mutagenicity tests on organic chemical contaminants in city water and related compounds. II. Chromosome aberration tests in cultured mammalian cells] Kokuritsu Iyakuhin Shokuhin Eisei Kenkyu¯jo Ho¯koku / Bulletin of the National Institute of Health Sciences, 103(103,103), 64-75
Data Type:	Chromosomal aberrations_Perc
HERO ID:	201741

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 20:	Confounding Variables in Test Design and Procedures	Not Rated	NA	NA	Study details are not available because it is a foreign language study; only the abstract and data tables are provided in English.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Not Rated	NA	NA	Study details are not available because it is a foreign language study; only the abstract and data tables are provided in English.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	The abstract of the study cites "significant" test results (significant increase in aberrations for other chemicals tested). However, information on statistical analyses (if performed) are not available because it is a foreign language study; only the abstract and data tables are provided in English.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	The results were judged to be positive, negative, or equivocal in the data tables; however, details with respect to the evaluation criteria are not available because it is a foreign language study; only the abstract and data tables are provided in English.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity was assessed (because the data tables indicate concentrations at which there was almost no survival of cells). However, methods utilized in the assessment of cytotoxicity were not available; only the abstract and data tables are provided in English.
	Metric 25:	Reporting of Data	High	× 2	2	Data were provided for all outcomes by exposure group.
Overall Quality Determination‡			~~High~~ ⟶ Low§		~~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

§ Evaluator's explanation for rating change: "Few study details are available. The study is in Japanese; only the abstract and data tables are provided in English."

Table 64: **In vitro evaluation results of Hasspieler et al 2006 for DNA SSBs and repair**

Study Citation:	Hasspieler, B., Haffner, D., Stelljes, M., Adeli, K. (2006). Toxicological assessment of industrial solvents using human cell bioassays: assessment of short-term cytotoxicity and long-term genotoxicity potential Toxicology and Industrial Health, 22(7,7), 301-315
Data Type:	DNA SSBs and repair for PCE
HERO ID:	478653

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name, CASRN, and structural formula.
	Metric 2:	Test Substance Source	High	× 1	1	The test substance source (a manufacturer) was reported. Although a batch/lot number were not reported, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	Low	× 1	3	The test substance purity/grade was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using negative (solvent-only) controls. The study indicated that DMSO and acetone were used; however, the solvent used for perc was not explicitly specified.
	Metric 5:	Positive Controls	High	× 2	2	The study authors reported using a positive control for the DNA damage and repair assays (4-nitroquinoline N-oxide).
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods/procedures were described, but specific details were not reported (e.g., volumes). It was indicated that the procedure used for analyzing DNA SSB assay was a modification of a procedure cited to another publication (Hasspieler et al. 1995).
	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	It was indicated that the test substance was dissolved in solvent. Storage was not reported (but it not expected to impact the study results given the short-term nature of the experiments).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration appeared to be consistent across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	A range of doses tested was reported (25 to 500 ppm).

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Study Citation:	Hasspieler, B., Haffner, D., Stelljes, M., Adeli, K. (2006). Toxicological assessment of industrial solvents using human cell bioassays: assessment of short-term cytotoxicity and long-term genotoxicity potential Toxicology and Industrial Health, 22(7,7), 301-315
Data Type:	DNA SSBs and repair for PCE
HERO ID:	478653

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	The exposure duration for other assays performed in the study were up to 24 hours (cytotoxicity) or 24 hours (EROD bioassay). Descriptions of the genotoxicity assays (DNA SSB and repair assays) reported treatments "for a given period of time," and reference information described above for other assay types. The duration of exposure for the genotoxicity assays was not explicitly specified (DNA SSB duration may be included in a cited publication and/or 24 hours may be presumed). Based on positive results (e.g., for the positive control), the exposure duration was presumably adequate for the outcome of interest.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of exposure groups was not reported (presumably similar of the same as the doses used for other chemicals tested in the study). A rationale for dose selection was suggested (similar to expected tissue concentrations); however, the doses used for perc caused substantial toxicity (significant at all doses based on Table 2).
	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 (human HepG2 cells) was reported and is routinely used for toxicity studies. The source of the cell line was specified, but few details were provided.
	Metric 15:	Number per Group	Low	× 1	3	The number of replicates used for perc were not reported, but were assumed to be similar to the number used for other chemicals tested in the same study (n = 4).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methods were described and appeared appropriate for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessments appeared to be consistent 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	Low	× 2	6	Test design or procedural confounding variables were not reported.

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Study Citation:	Hasspieler, B., Haffner, D., Stelljes, M., Adeli, K. (2006). Toxicological assessment of industrial solvents using human cell bioassays: assessment of short-term cytotoxicity and long-term genotoxicity potential Toxicology and Industrial Health, 22(7,7), 301-315
Data Type:	DNA SSBs and repair for PCE
HERO ID:	478653

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables in health outcomes unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	It was indicated that statistical analyses were performed (threshold $p < 0.05$); however, details of tests conducted were not provided. Data provided are not amenable to independent analyses.
	Metric 23:	Data Interpretation	High	× 2	2	Based on information provided in Table 2, a test was scored as positive when percent change in activity was statistically significantly different from the negative control.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity methods were described; these methods (neutral red uptake assay) are commonly used.
	Metric 25:	Reporting of Data		× 2	NA	Data were summarized in Table 2 (as positive for SSBs and negative for repair based on statistical significance). However, the supporting data were not shown.
Overall Quality Determination‡			Unacceptable⋆⋆		1.9	
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 65: **In vitro evaluation results for Emmert et al 2006 for bacterial reverse mutation study**

Study Citation:	B. Emmert, J. Bünger, K. Keuch, M. Müller, S. Emmert, E. Hallier, G. A. Westphal (2006). Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S. typhimurium strain YG7108pin3ERb5 Toxicology, 228(1,1), 66-76
Data Type:	Bacterial reverse mutation for perc
HERO ID:	597695

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as tetrachloroethylene. A CASRN was also provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported. An analysis number was also provided.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be at least 99.5% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	The study indicated that the test substance was tested as a solution in DMSO. However, the legend for Figure 8 states microcolony induction by the test substance (10 to 25 ug/uL in ethanol). There is uncertainty as to the vehicle-control substance that was used.
	Metric 5:	Positive Controls	High	× 2	2	Concurrent positive controls (N-Nitrosodiethylamine) were included in the experimental design. Positive controls yielded positive results.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were described and partially cited to another publication. The study indicated that the Ames test was carried out according to Maron and Ames (1983) with slight modifications owing to the bacterial strain that was used in 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	High	× 1	1	Test substance preparation was reported. Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (i.e., could be estimated from data shown in Figure 8).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate. The authors provided a justification for an extended exposure time (i.e., the strain grows slowly in the presence of toxicants).

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Study Citation:	B. Emmert, J. Bünger, K. Keuch, M. Müller, S. Emmert, E. Hallier, G. A. Westphal (2006). Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S. typhimurium strain YG7108pin3ERb5 Toxicology, 228(1,1), 66-76
Data Type:	Bacterial reverse mutation for perc
HERO ID:	597695

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups was reported (can be ascertained based on data shown in Figure 8). The study indicated that test substances were initially tested up to 5 mg/plate, toxic concentrations, or the highest soluble concentration (to determine the concentration range for the mutagenicity assay).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type. Conventional S9 activation was used for some assays (but not for this test substance). The bacterial strain used in this assay conferred metabolic competence (including CYP P450 2E1).
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was provided with some descriptive information. The strain appeared to be laboratory-maintained;the strain had to be transformed with a plasmid for each test (because large plasmids are often lost). The strain has not been routinely used in studies of this type.
	Metric 15:	Number per Group	High	× 1	1	Each experimental condition was conducted 5 times.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The outcome assessment methodology (numbers of revertant colonies) is routinely used for the outcome of interest. However, the sensitivity of the assay to detect an effect is uncertain (the authors indicated that cytototoxic metabolites were produced by the metabolically-competent bacterial strain used in the assay). The study states that either the metabolites generated by the strain were not mutagenic, the strain is not sensitive for these compounds, or the bacteria masks possible mutagenic effects.
	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. Colony counting was conducted automatically.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were reported.

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Study Citation:	B. Emmert, J. Bünger, K. Keuch, M. Müller, S. Emmert, E. Hallier, G. A. Westphal (2006). Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S. typhimurium strain YG7108pin3ERb5 Toxicology, 228(1,1), 66-76
Data Type:	Bacterial reverse mutation for perc
HERO ID:	597695

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	The study does not indicate that statistical analysis was conducted; this analysis is not required by study type (fold-changes relative to control are evaluated). Data were presented as means +/- standard deviations.
	Metric 23:	Data Interpretation	High	× 2	2	The study clearly specified the criteria for a positive result. Results were considered positive if at least 2 consecutive doses were 2x baseline with dose-dependency.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Not required by study type. The study eluded to preliminary toxicity testing to define the dose range (not further described).
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group for microcolony induction (indicative of toxicity). Data for mutagenicity were qualitative (indicated as negative).
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 66: **In vitro evaluation results for von der Hude et al 1988 for bacterial mutagenicity study**

Study Citation:	W. von der Hude, C. Behm, R. Gürtler, A. Basler (1988). Evaluation of the SOS chromotest Mutation Research, 203(2,2), 81-94
Data Type:	Perc SOS chromotest in E coli PQ37
HERO ID:	627708

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	test substance reported by name and CAS
	Metric 2:	Test Substance Source	Low	× 1	3	test substance source 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) control was reported
	Metric 5:	Positive Controls	High	× 2	2	concurrent positive controls were included in the presence (BaP) and absence (4-NQO) of metabolic activation
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were previously cited, and briefly reported and appropriate for the study
	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	Medium	× 1	2	test substance storage was not reported but is unlikely to impact this short duration study. Preparation was inferred (dissolved in solvent)
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	exposure methods were briefly described and cited to previous publication
	Metric 10:	Reporting of Doses/Concentrations	Unacceptable	× 2	8	Concentrations were not specified; reported in methods as 3-5 concentrations at half log intervals up to the limit of solubility or 100 mM
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	exposure duration was 2h incubation period and was adequate for the study type
	Metric 12:	Exposure Route and Method	High	× 1	1	number of exposure groups (3-5) and spacing (half-log intervals) was consistent with standards; tested up to solubility limit or 100 mM
	Metric 13:	Metabolic Activation	Medium	× 1	2	metabolic activation was reported, commonly used, and details were cited to other publications
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Test model (E. coli PQ37) was reported with limited descriptive information. It is routinely used for the outcome of interest. The test model was not obtained from a commercial source but a private individual.

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Study Citation: W. von der Hude, C. Behm, R. Gürtler, A. Basler (1988). Evaluation of the SOS chromotest Mutation Research, 203(2,2), 81-94
Data Type: Perc SOS chromotest in E coli PQ37
HERO ID: 627708

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 15:	Number per Group	Medium	× 1	2	Optical density of experimental cultures was reported and consistent across groups. Study reports validation of results in independent assays (n not reported)
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	outcome assessment methodology (SOS chromotest) was described and appeared appropriate for the study
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	outcome assessment was carried out consistently across groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	not applicable for the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	not applicable for the study type
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	statistical analysis was not described but is not necessary for this outcome
	Metric 23:	Data Interpretation	High	× 2	2	evaluation criteria were reported (considered a result to be positive only if SOS induction factor increase over control was more than 0.5 AND increasing beta-Gal activity was observed) and more rigorous than standard practice at the time
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Alkaline phosphatase portion of assay is a measure of cytotoxicity; however, results were not reported for test chemical
	Metric 25:	Reporting of Data	Low	× 2	6	Results were reported qualitatively and in summary form in Table 3
Overall Quality Determination‡			Unacceptable**		1.8	
Extracted			No			

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Study Citation:	W. von der Hude, C. Behm, R. Gürtler, A. Basler (1988). Evaluation of the SOS chromotest Mutation Research, 203(2,2), 81-94
Data Type:	Perc SOS chromotest in E coli PQ37
HERO ID:	627708

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 67: **In vitro evaluation results for Demarini et al 1994 for bacterial reverse mutation study**

Study Citation:	D. M. Demarini, E. Perry, M. L. Shelton (1994). Dichloroacetic acid and related compounds: Induction of prophage in E. coli and mutagenicity and mutation spectra in Salmonella TA100 Mutagenesis, 9(5,5), 429-437
Data Type:	Reverse mutation for PERC and metabolites (TCA, TCAC)
HERO ID:	628757

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	test substances were reported by name, CASRN, and molecular weight
	Metric 2:	Test Substance Source	High	× 1	1	test substance source (Sigma) was reported, batch/lot was not reported but composition is not expected to vary
	Metric 3:	Test Substance Purity	High	× 1	1	Purity of all chemicals was reported to be 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	concurrent negative controls were used, but it is unclear if they were untreated or vehicle controls.
	Metric 5:	Positive Controls	High	× 2	2	concurrent positive controls (sodium azide without S9 and 2-AA with S9) were used with and without metabolic activation
	Metric 6:	Assay Procedures	Medium	× 1	2	assay procedures were cited to a prior publication, and modifications were described and appeared appropriate
	Metric 7:	Standards for Tests	Not Rated	NA	NA	not applicable for the study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was accomplished by injection into the sealed bag . Storage was not reported but is unlikely to impact this short term study.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposure methods were cited to a prior publication and briefly described and appeared to be consistent across groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	concentrations were reported in figure 3 (in mg/ml) and can be estimated/quantified
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	exposure duration was 24h and appears to be adequate for the study
	Metric 12:	Exposure Route and Method	High	× 1	1	concentrations (4 plus control) and spacing were reported; high concentration justified by authors as up to cytotoxic doses
	Metric 13:	Metabolic Activation	High	× 1	1	metabolic activation was reported and commonly used; preparation was cited to another publication
Domain 4: Test Model						

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Study Citation:	D. M. Demarini, E. Perry, M. L. Shelton (1994). Dichloroacetic acid and related compounds: Induction of prophage in E. coli and mutagenicity and mutation spectra in Salmonella TA100 Mutagenesis, 9(5,5), 429-437
Data Type:	Reverse mutation for PERC and metabolites (TCA, TCAC)
HERO ID:	628757

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	Test model (S typhimurium strain TA100) was briefly characterized and is appropriate for the study type. Test model was not obtained from commercial source but from private researcher. Specific single strain was selected with justification for evaluation of specific revertant codon mutation
	Metric 15:	Number per Group	Medium	× 1	2	Each experiment performed at least twice
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology (colony counting) was reported (Automatic colony counter) and appropriate
	Metric 17:	Consistency of Outcome Assessment	Low	× 1	3	Consistent outcome assessment across groups is inferred from the text
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	not applicable for the study
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	not applicable for the study
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	statistical analysis was not performed but is not required for this study type
	Metric 23:	Data Interpretation	High	× 2	2	Criterion for a positive response was a reproducible 2-fold increase in revertants/plate over background and is consistent with standard practice
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were defined, but the methods of measurements were not fully described or reported
	Metric 25:	Reporting of Data	High	× 2	2	Results reported for each concentration and each experiment as a mean and SEM of duplicate plates
Overall Quality Determination‡			High		1.5	
Extracted			Yes			

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Study Citation:	D. M. Demarini, E. Perry, M. L. Shelton (1994). Dichloroacetic acid and related compounds: Induction of prophage in E. coli and mutagenicity and mutation spectra in Salmonella TA100 Mutagenesis, 9(5,5), 429-437
Data Type:	Reverse mutation for PERC and metabolites (TCA, TCAC)
HERO ID:	628757

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 68: **In vitro evaluation results for Demarini et al 1994 for bacterial DNA damage study**

Study Citation:	D. M. Demarini, E. Perry, M. L. Shelton (1994). Dichloroacetic acid and related compounds: Induction of prophage in E. coli and mutagenicity and mutation spectra in Salmonella TA100 Mutagenesis, 9(5,5), 429-437
Data Type:	DNA damage (prophage induction) for PERC and metabolites (TCA, TCAC)
HERO ID:	628757

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	test substances were reported by name, CASRN, and molecular weight
	Metric 2:	Test Substance Source	High	× 1	1	test substance source (Sigma) was reported, batch/lot was not reported but composition is not expected to vary
	Metric 3:	Test Substance Purity	High	× 1	1	Purity of all chemicals was reported to be 99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	concurrent negative controls (media) were reported
	Metric 5:	Positive Controls	High	× 2	2	concurrent positive controls (2-nitrofluorene without S9 and 2-aminoanthracine with S9) were used
	Metric 6:	Assay Procedures	Medium	× 1	2	assay procedures were cited to a prior publication, briefly described and appeared appropriate for the study type
	Metric 7:	Standards for Tests	Not Rated	NA	NA	not applicable for the study
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of the test substance was a dilution series in medium. Storage was not reported but is unlikely to impact this short term study.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposure methods were cited to a prior publication and briefly described and appeared to be consistent across groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	concentrations were reported in figure 2 (in mg/ml) and can be estimated/quantified
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	exposure duration was an overnight incubation, not further described but appeared to be appropriate for the study type
	Metric 12:	Exposure Route and Method	High	× 1	1	concentrations (4 plus control) and spacing were reported; high concentration justified by authors as up to cytotoxic doses
	Metric 13:	Metabolic Activation	High	× 1	1	metabolic activation was reported and commonly used; preparation was cited to another publication
Domain 4: Test Model						

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Study Citation:	D. M. Demarini, E. Perry, M. L. Shelton (1994). Dichloroacetic acid and related compounds: Induction of prophage in E. coli and mutagenicity and mutation spectra in Salmonella TA100 Mutagenesis, 9(5,5), 429-437
Data Type:	DNA damage (prophage induction) for PERC and metabolites (TCA, TCAC)
HERO ID:	628757

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	Test model (E coli) was briefly characterized and is appropriate for the study type. Test model was not obtained from commercial source but from private researcher.
	Metric 15:	Number per Group	Medium	× 1	2	Each experiment performed at least twice
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	outcome assessment methodology (hand counting of plaque forming units) was described and appeared appropriate for the outcome of interest
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Consistent outcome assessment across groups is inferred from the text
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	not applicable for the study
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	not applicable for the study
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Low	× 1	3	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	statistical analysis was not performed but is not required for this study type
	Metric 23:	Data Interpretation	High	× 2	2	Criterion for a positive response was 3-fold increase in PFU/plate over background and reproducible dose dependent increase and is consistent with standards and previous citations
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were defined, but the methods of measurements were not fully described or reported
	Metric 25:	Reporting of Data	High	× 2	2	Results reported for each concentration and each experiment as a mean and SEM of duplicate plates
Overall Quality Determination‡			High		1.4	
Extracted			Yes			

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Study Citation:	D. M. Demarini, E. Perry, M. L. Shelton (1994). Dichloroacetic acid and related compounds: Induction of prophage in E. coli and mutagenicity and mutation spectra in Salmonella TA100 Mutagenesis, 9(5,5), 429-437
Data Type:	DNA damage (prophage induction) for PERC and metabolites (TCA, TCAC)
HERO ID:	628757

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 69: **In vitro evaluation results for Dreessen et al 2003 for Ames test study**

Study Citation:	B. Dreessen, G. Westphal, J. Bünger, E. Hallier, M. Müller (2003). Mutagenicity of the glutathione and cysteine S-conjugates of the haloalkenes 1,1,2-trichloro-3,3,3-trifluoro-1-propene and trichlorofluoroethene in the Ames test in comparison with the tetrachloroethene-analogues Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 539(1-2,1-2), 157-166
Data Type:	Perc Metabolites (TCVC and TCVG) Ames Test
HERO ID:	628759

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Perc metabolites were identified by chemical name (S-(1,2,2-trichlorovinyl)-l-cysteine (TCVC) and S-(1,2,2-trichlorovinyl)glutathione (TCVG)).
	Metric 2:	Test Substance Source	High	× 1	1	TCVC was synthesized according to the procedures of Moore and Green, 1988 and TCVG was synthesized by dropwise addition of tetrachloroethene to a solution of L-glutathione and 1,5-diazabicyclo[4.3.0]non-5-ene in dry dimethylformamide under nitrogen. Synthesized products were purified by preparative HPLC.
	Metric 3:	Test Substance Purity	High	× 1	1	HPLC determined purities were at least 98%.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Low	× 2	6	Negative controls are not described but were indicated in Figures 3 and 4 as a 0 mmol/L plate for TA100. It is unclear whether the negative controls were solvent or untreated controls. A control for TA98 was presumed based on the similar summarized results reported, but it was not specified.
	Metric 5:	Positive Controls	High	× 2	2	TCVC and TCVG were considered the positive controls in this experiment. DCTFPC and DCFVC were also tested and were considered mutagenic, just at higher concentrations. The system was capable of detecting a positive response.
	Metric 6:	Assay Procedures	Medium	× 1	2	The Ames test was carried out without preincubation according to Maron and Ames, 1983 (Revised method for the Salmonella mutagenicity test). Methods were briefly described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						

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Study Citation:	B. Dreessen, G. Westphal, J. Bünger, E. Hallier, M. Müller (2003). Mutagenicity of the glutathione and cysteine S-conjugates of the haloalkenes 1,1,2-trichloro-3,3,3-trifluoro-1-propene and trichlorofluoroethene in the Ames test in comparison with the tetrachloroethene-analogues Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 539(1-2,1-2), 157-166
Data Type:	Perc Metabolites (TCVC and TCVG) Ames Test
HERO ID:	628759

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Preparation of test substance was described – haloalkene cysteine S-conjugates were dissolved in 50% ethanol and haloalkene glutathione S-conjugates were dissolved in DMSO. Stock solutions of 10 mM TCVC and 10 mM TCVG were used. Mixtures were vortexed. Plate-incorporation was used, and might not be capable of accounting for volatility. The short duration of this study (48 hours) did not require storage details.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure appears consistent across dose groups. Study performed according to Maron and Ames, 1983 (Revised method for the Salmonella mutagenicity test). Methods were briefly described.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Exposure concentrations can be determined from Figures 3 (TCVC) and 4 (TCVG) for TA100 only; a graph of results for TA98 was not provided, although similar assay methods were used for TA98 and TA100 with TCVC.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Plates were incubated for 48 hours.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Figure 4 shows that 5 or more concentrations were tested for TA100 and the compounds were tested up to their solubility limits or to toxic concentrations. Only 1-2 Salmonella strains were used in each experiment and the results for TA98 were not provided for each concentration (only summarized).
	Metric 13:	Metabolic Activation	High	× 1	1	Arochlor-1254 induced Sprague-Dawley rat kidney S9-protein fractions was used for metabolic activation. Rats were purchased from Organon Teknika (Tournhout, Belgium), kidneys were homogenized and then frozen in nitrogen. 500 uL of the kidney S9 was used for metabolic activation only for TA100 with TCVG. TCVC (TA98 and TA100) was tested without metabolic activation, however, it was noted that metabolic activation was not required for the haloalkene cysteine S-conjugates because 'both strains express high activities of bacterial B-lyase'.
Domain 4: Test Model						

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Study Citation:	B. Dreessen, G. Westphal, J. Bünger, E. Hallier, M. Müller (2003). Mutagenicity of the glutathione and cysteine S-conjugates of the haloalkenes 1,1,2-trichloro-3,3,3-trifluoro-1-propene and trichlorofluoroethene in the Ames test in comparison with the tetrachloroethene-analogues Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 539(1-2,1-2), 157-166
Data Type:	Perc Metabolites (TCVC and TCVG) Ames Test
HERO ID:	628759

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	Haloalkene cysteine S-conjugates were tested with S. typhimurium strains TA98 and TA100 and haloalkene glutathione S-conjugates were tested with TA100 only. Only 1-2 strains were tested per test substance in an Ames test. It is unclear if these strains were from a commercial source or laboratory-maintained.
	Metric 15:	Number per Group	High	× 1	1	'Two independent sets of experiments were performed, each in duplicate'. Study was also performed according to Maron and Ames, 1983 (Revised method for the Salmonella mutagenicity test).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Study was performed according to Maron and Ames, 1983 (Revised method for the Salmonella mutagenicity test). Revertant rates and dose-response were evaluated.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Study was performed according to Maron and Ames, 1983 (Revised method for the Salmonella mutagenicity test). No inconsistencies were reported and consistency appeared appropriate. However, details results were not provided for TA98 tested with TCVC.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design (Ames mutagenicity assay).
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design, as no subjective outcomes were assessed.
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 outcome differences unrelated to exposure were not reported for each study replicate or group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Medium	× 1	2	Statistical analysis was not performed; however, standard deviations and individual results were reported in Figured 3 and 4 for TA100 (not TA98). Independent analysis is possible for TA100 only. Dose-response was examined for both TA98 and TA100 for both test substances.

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Study Citation:	B. Dreessen, G. Westphal, J. Bünger, E. Hallier, M. Müller (2003). Mutagenicity of the glutathione and cysteine S-conjugates of the haloalkenes 1,1,2-trichloro-3,3,3-trifluoro-1-propene and trichlorofluoroethene in the Ames test in comparison with the tetrachloroethene-analogues Mutation Research: Genetic Toxicology and Environmental Mutagenesis, 539(1-2,1-2), 157-166
Data Type:	Perc Metabolites (TCVC and TCVG) Ames Test
HERO ID:	628759

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria was briefly described - dose response and a five-fold revertant rate over the background was appropriate for the positive controls, which were TCVC and TCVG. Study was performed according to Ames.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Compounds were tested up to their solubility limits or to toxic concentrations; however, cytotoxic concentrations were not reported.
	Metric 25:	Reporting of Data	Medium	× 2	4	Individual results were reported for TCVC without metabolic activation (not required) with TA100 and for TCVG with TA100 with and without metabolic activation. Summarized results were provided for TA98 without metabolic activation, but no graph was provided.
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 70: **In vitro evaluation results for Koch et al 1988 for S. cerevisiae reverse mutation study**

Study Citation:	R. Koch, R. Schlegelmilch, H. U. Wolf (1988). Genetic effects of chlorinated ethylenes in the yeast Saccharomyces cerevisiae Mutation Research, 206(2,2), 209-216
Data Type:	Perc mitotic gene conversion, reverse mutation and aneuploidy in yeast
HERO ID:	628846

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test material was identified by chemical name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified. Batch/lot number were not given, but the composition of the test material is not expected to vary.
	Metric 3:	Test Substance Purity	High	× 1	1	Analytical grade.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors acknowledged using a concurrent negative control group, but details regarding the negative control group were not reported. However, because test substances were pipetted directly into cell suspensions without vehicle, it is assumed that negative controls were untreated.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls (EMS) were used and responded appropriately.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described in detail and applicable to the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	The test substance was added without dilution to the cell suspensions. This is considered to add uncertainty to the dosing, as direct dilution is less accurate than serial dilution due to human error or mechanical considerations (e.g. multiple pipettes used and potentially not calibrated appropriately).
	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 are reported as mM without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appropriate for the study type and outcome.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Concentrations were not justified, only 2 groups were used (plus control). Excess cytotoxicity was observed in the high dose group.

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Study Citation:	R. Koch, R. Schlegelmilch, H. U. Wolf (1988). Genetic effects of chlorinated ethylenes in the yeast Saccharomyces cerevisiae Mutation Research, 206(2,2), 209-216
Data Type:	Perc mitotic gene conversion, reverse mutation and aneuploidy in yeast
HERO ID:	628846

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
	Metric 13:	Metabolic Activation	High	× 1	1	Metabolic acrivation systems were well described.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was described with limited information (details cited elsewhere) and was routinely used.
	Metric 15:	Number per Group	Medium	× 1	2	Duplicate independent assays.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment reported and was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences were reported in initial conditions.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No differences were reported in the test model unrelated to exposure.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistics were not performed, but may not be necessary . Given values were from 1 representative test.
	Metric 23:	Data Interpretation	High	× 2	2	Scoring and/or evaluation criteria (i.e. meaning of colony colors and which were counted) were adequately reported.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were defined, but the methods of measurements were not fully described or reported.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes by exposure group. Negative findings were reported quantitatively.
Overall Quality Determination[‡]			~~High~~ ⟶ Low[§]		~~1.3~~	
Extracted			No			

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Study Citation:	R. Koch, R. Schlegelmilch, H. U. Wolf (1988). Genetic effects of chlorinated ethylenes in the yeast Saccharomyces cerevisiae Mutation Research, 206(2,2), 209-216
Data Type:	Perc mitotic gene conversion, reverse mutation and aneuploidy in yeast
HERO ID:	628846

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: "Perc was highly toxic to yeast, precluding an evaluation of genotoxicity in this test system."

Table 71: **In vitro evaluation results for Perocco et al 1983 for human lymphocyte unscheduled DNA synthesis study**

Study Citation:	P. Perocco, S. Bolognesi, W. Alberghini (1983). Toxic activity of seventeen industrial solvents and halogenated compounds on human lymphocytes cultured in vitro Toxicology Letters, 16(1-2,1-2), 69-75
Data Type:	UDS assay in human lymphocytes for perc
HERO ID:	628879

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as tetrachloroethylene.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported (Aldrich Europe).
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported (99%).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using a concurrent negative (vehicle-only) control. In addition, chloroform was considered a negative control substance.
	Metric 5:	Positive Controls	Low	× 2	6	Chloromethyl methyl ether was considered a positive control substance (not clear if run concurrently with all test substances). The study indicated that this test substance was positive for DNA synthesis in the presence of activation (criteria for positive response not clearly specified); a positive control substance without activation was not specified.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were briefly described.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Test substance preparation was described (i.e., diluted in DMSO). The test substance was prepared before addition to cell cultures. Storage was not reported (but not expected to impact study results).
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposures were inferred to be administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity in Table 1.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (4 hours) and considered appropriate for the study type.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure concentrations were reported (3 scalar doses). A rationale for dose selection was not provided.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Rat liver phenobarbital-induced S9 mix was used; this was obtained following the methods of Ames et al. (1975). Composition of the S9 mix was reported.

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Study Citation: P. Perocco, S. Bolognesi, W. Alberghini (1983). Toxic activity of seventeen industrial solvents and halogenated compounds on human lymphocytes cultured in vitro Toxicology Letters, 16(1-2,1-2), 69-75
Data Type: UDS assay in human lymphocytes for perc
HERO ID: 628879

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model (human lymphocytes) was reported with limited details (i.e., from the blood of healthy donors). The test system was cited to other publications (Rocchi et al., 1980; Perocco and Prodi, 1981).
	Metric 15:	Number per Group	High	× 1	1	The number of cells were reported. It was indicated that sextuplicate samples were used.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported and appropriate for the endpoints of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	The outcome assessment was inferred to be carried out consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to the study type.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable to the study type.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Initial quality of tissues exposed or lot of test substance was not reported. It is noted that repeated experiments used cells from different donors to confirm results.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were not used; however, results were presented as means +/- standard error for sextuplicate samples (i.e., data are amenable to independent statistical analysis).
	Metric 23:	Data Interpretation	Low	× 2	6	Evaluation criteria were not reported.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were defined (i.e., trypan blue exclusion); however, methods were not fully described or reported.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group.
Overall Quality Determination‡			Medium		1.7	
Extracted			Yes			

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Study Citation:	P. Perocco, S. Bolognesi, W. Alberghini (1983). Toxic activity of seventeen industrial solvents and halogenated compounds on human lymphocytes cultured in vitro Toxicology Letters, 16(1-2,1-2), 69-75
Data Type:	UDS assay in human lymphocytes for perc
HERO ID:	628879

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 72: **In vitro evaluation results for Hartmann and Speit 1995 for sister chromatid exchange study**

Study Citation:	A. Hartmann, G. Speit (1995). Genotoxic effects of chemicals in the single cell gel (SCG) test with human blood cells in relation to the induction of sister-chromatid exchanges (SCE) Mutation Research Letters, 346(1,1), 49-56
Data Type:	DNA damage and SCEs in human white blood cells for Perc
HERO ID:	628891

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as tetrachloroethylene (PER).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported (Aldrich).
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of test substance was not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using concurrent negative controls. Based on the statement in the results that indicated that under conditions using S9 and DMSO as a solvent, baseline migration was increased, the negative control was presumably solvent-only (rather than untreated).
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not used; however, test substances used in the study produced positive results demonstrating that the test is capable of detecting a positive response.
	Metric 6:	Assay Procedures	High	× 1	1	Study authors described the methods and procedures used for the test and they were applicable for the study type. It was noted that the DNA migration test was performed as described by Singh et al. (1988) with minor modifications.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for this study type.
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 reported for the short-term studies but is unlikely to affect results.
	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	Doses were reported in the tables and figures.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration was reported (2 hours for the DNA migration test; 2 or 24 hours for the SCE test). The duration of the DNA migration test was slightly shorter than recommended by study type (and negative results were observed).

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Study Citation:	A. Hartmann, G. Speit (1995). Genotoxic effects of chemicals in the single cell gel (SCG) test with human blood cells in relation to the induction of sister-chromatid exchanges (SCE) Mutation Research Letters, 346(1,1), 49-56
Data Type:	DNA damage and SCEs in human white blood cells for Perc
HERO ID:	628891

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of doses was reported (3 plus controls for DNA migration and 4 plus controls for SCEs). Although a rationale for dose spacing was not provided, perc was tested at up to cytotoxic concentrations.
	Metric 13:	Metabolic Activation	High	× 1	1	Exposures were conducted in the presence and absence of a metabolic activation system (Aroclor 1254-induced S9 liver fraction from CCR, Robdorf, Germany). Preparation of S9 mix was described.
Domain 4: Test Model						
	Metric 14:	Test Model	Low	× 2	6	The test model was reported with no additional information. The test model was routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	The study indicated that replicate slides were used for the DNA migration study; for SCEs, all experiments were repeated in independent trials.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was reported and 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	High	× 2	2	For the DNA migration study, 50 cells were analyzed (25 cells from each of two replicates); this number is consistent with recommendations for this study type. For SCEs, 100 metaphases were evaluated.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This method 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 quality of tissues exposed or lot of test substance was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was conducted on results using one-tailed t-tests.
	Metric 23:	Data Interpretation	Medium	× 2	4	Evaluation criteria were not explicitly reported. Based on information provided in the results, statistical significance and/or dose-relatedness were the criteria for a positive response.

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Study Citation:	A. Hartmann, G. Speit (1995). Genotoxic effects of chemicals in the single cell gel (SCG) test with human blood cells in relation to the induction of sister-chromatid exchanges (SCE) Mutation Research Letters, 346(1,1), 49-56
Data Type:	DNA damage and SCEs in human white blood cells for Perc
HERO ID:	628891

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 24:	Cytotoxicity Data	Medium	× 1	2	Cytotoxicity endpoints were described (viability). For the DNA migration test, cell viability was measured (shown in Figure 2). For the SCE test, the study authors reported the proliferation index.
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcomes were presented for each exposure group (with and without metabolic activation and time points) as a mean and standard error.
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 73: **In vitro evaluation results for Mazzullo et al 1987 for DNA binding study**

Study Citation:	M. Mazzullo, S. Grilli, G. Lattanzi, G. Prodi, M. P. Turina, A. Colacci (1987). Evidence of DNA binding activity of perchloroethylene Research Communications in Chemical Pathology and Pharmacology, 58(2,2), 215-235
Data Type:	In vitro binding to DNA and polynucleotides
HERO ID:	628902

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as 14C-Perchloroethylene (abbreviated [U-14C]-PCE).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported as The Radiochemical Centre, Amersham, England.
	Metric 3:	Test Substance Purity	Medium	× 1	2	Radiochemical purity of the test substance was 97%. PCE impurity was due to hexachloroethane utilized in its synthesis. It was unclear whether any hexachloroethane was radiolabeled. Hexachloroethane has been previously linked to DNA binding (Lattanzi et al. 1987).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Not Rated	NA	NA	This metric is not applicable to this study type (DNA binding).
	Metric 5:	Positive Controls	Not Rated	NA	NA	This metric is not applicable to this study type.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods were described, but some details were lacking (humidity, post-incubation conditions, etc.). It is assumed, but unclear, that the reaction conditions described were used for all of the in vitro assays (ex vivo and in vitro).
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	It is not clear whether the test compound was diluted or added neat to the incubation mixture.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	It appears the same methods of exposure were used.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	2.5 uCi of 14C-PCE was used.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Reported as 90 minutes for the standard incubation procedure.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	There was a single dose level in this study. The dose appeared to be adequate to assess the outcome of interest. Justification for the dose selection was not reported.

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Study Citation:	M. Mazzullo, S. Grilli, G. Lattanzi, G. Prodi, M. P. Turina, A. Colacci (1987). Evidence of DNA binding activity of perchloroethylene Research Communications in Chemical Pathology and Pharmacology, 58(2,2), 215-235
Data Type:	In vitro binding to DNA and polynucleotides
HERO ID:	628902

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	High	× 1	1	Metabolic activation systems included 2 mg microsomal protein (+2mg NADPH) and/or 6 mg cytosolic protein. In some experiments, microsomal and cytosolic fracteins were obtained from rats and mice pretreated with phenobarbital.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Calf thymus DNA and polyribonucleotides was used and obtained from Sigma Chemical Co. in St. Louis, MO.
	Metric 15:	Number per Group	High	× 1	1	Studies were performed in triplicate (Tables 2 and 4).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest – reports the specific activity of the DNA and polynucleotide interactions in pmol/mg.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently across groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the study design, as no subjective outcomes were assessed.
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 group.
	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 group.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was not required for this study type but was performed for the results in Tables 2 and 4. Additionally, enough information was provided to perform an independent statistical analysis.
	Metric 23:	Data Interpretation	High	× 2	2	DNA labeling was assayed using ultraviolet absorption measurement, specific colorimetric reactions, and counting in a Beckman LS-1801 liquid scintillation spectrometer.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	This metric is not applicable to the study design.
	Metric 25:	Reporting of Data	High	× 2	2	Results were provided for each group.
Overall Quality Determination‡			High		1.4	

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Study Citation:	M. Mazzullo, S. Grilli, G. Lattanzi, G. Prodi, M. P. Turina, A. Colacci (1987). Evidence of DNA binding activity of perchloroethylene Research Communications in Chemical Pathology and Pharmacology, 58(2,2), 215-235
Data Type:	In vitro binding to DNA and polynucleotides
HERO ID:	628902

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 74: **In vitro evaluation results for Vamvakas et al 1989 for unscheduled DNA synthesis study**

Study Citation:	Vamvakas, S., Dekant, W., Henschler, D. (1989). Assessment of unscheduled DNA synthesis in a cultured line of renal epithelial cells exposed to cysteine S-conjugates of haloalkenes and haloalkanes Mutation Research, 222(4,4), 329-335
Data Type:	Unscheduled DNA synthesis - TCVC (perc metabolite)
HERO ID:	629909

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as S-(1,2,2-trichlorovinyl)-L-cysteine (TCVC), a metabolite of Perc
	Metric 2:	Test Substance Source	Medium	× 1	2	The synthesis and characterization of S-(1,2,2-trichlorovinyl)-L-cysteine (TCVC) was described in previously published studies (Dekant et al., 1986; Vadi et al., 1985)
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of test substance was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors report using a medium and solvent (0.5% MeOH) control.
	Metric 5:	Positive Controls	High	× 2	2	Nitroquinoline oxide (NQO) was used as a positive control and gave expected results.
	Metric 6:	Assay Procedures	High	× 1	1	Study authors described the methods and procedures used for the test and they were applicable for the study type.
	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	High	× 1	1	Test substance preparation was described as dissolved in MeOH 30 to 60 seconds before incubation to avoid decomposition in solution.
	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 concentrations were reported in the results.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (24 hours).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of dose groups and spacing was not justified by the study authors, however the number of exposure groups and spacing were adequate to show results relative to the outcome of interest.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Not applicable
Domain 4: Test Model						

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Study Citation: Vamvakas, S., Dekant, W., Henschler, D. (1989). Assessment of unscheduled DNA synthesis in a cultured line of renal epithelial cells exposed to cysteine S-conjugates of haloalkenes and haloalkanes Mutation Research, 222(4,4), 329-335
Data Type: Unscheduled DNA synthesis - TCVC (perc metabolite)
HERO ID: 629909

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	The test model (LLC-PK1 cells) was reported with limited descriptive information. The cells were obtained from a commercial source (American Type Culture Collection). The test model is appropriate for the outcome of interest.
	Metric 15:	Number per Group	Medium	× 1	2	The number of cells was reported (2 x 10+6); Determinations made in quadruplicate and experiments were repeated at least 2 times.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were reported and 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	High	× 2	2	3x10+5 cells were plated on each culture dish determinations were made in quadruplicate and experiments were repeated at least 2 times.
	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	Low	× 2	6	Initial quality of cells exposed and lot of test substance was not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Significance of changes in UDS was noted; however, methods for statistical analysis were not clearly described; results shown in a figure indicate a mean and SD from 2 independent experiments; independent statistical analysis could be performed.
	Metric 23:	Data Interpretation	Low	× 2	6	Scoring and evaluation criteria were not reported; however, the induction of UDS is evaluated as a change from the control at 24 hours.
	Metric 24:	Cytotoxicity Data	High	× 1	1	There was a determination of cell viability as indicated by lactate dehydrogenase release in the medium.
	Metric 25:	Reporting of Data	High	× 2	2	Data for the outcomes were presented for each exposure group as a mean and SD.
Overall Quality Determination‡			High		1.5	

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Study Citation:	Vamvakas, S., Dekant, W., Henschler, D. (1989). Assessment of unscheduled DNA synthesis in a cultured line of renal epithelial cells exposed to cysteine S-conjugates of haloalkenes and haloalkanes Mutation Research, 222(4,4), 329-335
Data Type:	Unscheduled DNA synthesis - TCVC (perc metabolite)
HERO ID:	629909

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.

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

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

Table 75: **In vitro evaluation results for Vamvakas et al 1989 for unscheduled DNA synthesis study**

Study Citation:	S. Vamvakas, W. Dekant, D. Henschler (1989). Genotoxicity of haloalkene and haloalkane glutathione S-conjugates in porcine kidney cells Toxicology In Vitro, 3(2,2), 151-156
Data Type:	UDS for TCE metabolite (DCVG)
HERO ID:	629910

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The metabolite was clearly identified by name.
	Metric 2:	Test Substance Source	Low	× 1	3	The test substance was synthesized by the authors (according to Elfarra et a; 1986 and Vadi et al. 1985). Data on analytical verification were not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity/grade 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 concurrent (medium and solvent) negative controls.
	Metric 5:	Positive Controls	Low	× 2	6	The study authors reported using M-nitroquinoline oxide as a positive control; however, the control response was not described.
	Metric 6:	Assay Procedures	High	× 1	1	Methods and procedures were adequately described.
	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 test substance was reported. Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	It can be inferred from the study that exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (Figure 1).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The duration of exposure was reported. A rationale for the duration of exposure was provided (i.e., based on preliminary experiments of 3H-thy incorporation using DCVG).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of dose groups was reported (6 plus controls). In general, doses were adequate to evaluate dose-response relationships. The study indicated that lower concentrations were not cytotoxic; there was evidence of cytotoxicity at high doses.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type (metabolite was directly tested).
Domain 4: Test Model						

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Study Citation:	S. Vamvakas, W. Dekant, D. Henschler (1989). Genotoxicity of haloalkene and haloalkane glutathione S-conjugates in porcine kidney cells Toxicology In Vitro, 3(2,2), 151-156
Data Type:	UDS for TCE metabolite (DCVG)
HERO ID:	629910

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	The test model (LLC-PK1; porcine kidney cells) was obtained from commercial source; passage number was reported. Few other details were provided, and the cell type is not widely used in genotoxicity assays (cell type used because the test substances are nephrotoxic).
	Metric 15:	Number per Group	High	× 1	1	The number of replicates was reported (4 replicates and experiment repeated at least twice) and appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology were partially reported and cited elsewhere (Tsutsui et al. 1984).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	It is inferred from the text that the outcome was 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 (measurements were automated).
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Information on initial conditions for each study group are not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Information on study group differences unrelated to test substance are not fully reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Sufficient data were provided to conduct independent statistical analysis (presented as means +/- SD of 8 treated cultures from two independent experiments).
	Metric 23:	Data Interpretation	Low	× 2	6	The criteria for a positive response were not explicitly specified. Based on information in the results, it can be inferred that the dose-relatedness of the response was considered.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity data were included in evaluation. The study indicated that cytotoxicity was measured as LDH release from cells; these data were presented alongside the UDS data.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group (Figure 1).
Overall Quality Determination‡			Medium		1.7	

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Study Citation:	S. Vamvakas, W. Dekant, D. Henschler (1989). Genotoxicity of haloalkene and haloalkane glutathione S-conjugates in porcine kidney cells Toxicology In Vitro, 3(2,2), 151-156
Data Type:	UDS for TCE metabolite (DCVG)
HERO ID:	629910

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.

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

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

Table 76: **In vitro evaluation results for Vamvakas et al 1989 for unscheduled DNA synthesis study**

Study Citation:	S. Vamvakas, W. Dekant, D. Henschler (1989). Genotoxicity of haloalkene and haloalkane glutathione S-conjugates in porcine kidney cells Toxicology In Vitro, 3(2,2), 151-156
Data Type:	UDS for perc metabolite (TCVG)
HERO ID:	629910

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The metabolite was clearly identified by name.
	Metric 2:	Test Substance Source	Low	× 1	3	The test substance was synthesized/characterized by the authors (according to Dekant et al. 1987, 1988). Data on analytical verification were not reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity/grade 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 concurrent (medium and solvent) negative controls.
	Metric 5:	Positive Controls	Low	× 2	6	The study authors reported using M-nitroquinoline oxide as a positive control; however, the control response was not described.
	Metric 6:	Assay Procedures	High	× 1	1	Methods and procedures were adequately described.
	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 test substance was reported. Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	It can be inferred from the study that exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity (Figure 1).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The duration of exposure was reported. A rationale for the duration of exposure was provided (i.e., based on preliminary experiments of 3H-thy incorporation using DCVG).
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of dose groups was reported (6 plus controls). In general, doses were adequate to evaluate dose-response relationships. The study indicated that lower concentrations were not cytotoxic; there was evidence of cytotoxicity at high doses.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	This metric is not applicable to the study type (metabolite was directly tested).
Domain 4: Test Model						

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Study Citation: S. Vamvakas, W. Dekant, D. Henschler (1989). Genotoxicity of haloalkene and haloalkane glutathione S-conjugates in porcine kidney cells Toxicology In Vitro, 3(2,2), 151-156
Data Type: UDS for perc metabolite (TCVG)
HERO ID: 629910

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	The test model (LLC-PK1; porcine kidney cells) was obtained from commercial source; passage number was reported. Few other details were provided, and the cell type is not widely used in genotoxicity assays (cell type used because the test substances are nephrotoxic).
	Metric 15:	Number per Group	High	× 1	1	The number of replicates was reported (4 replicates and experiment repeated at least twice) and appropriate for the study type.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology were partially reported and cited elsewhere (Tsutsui et al. 1984).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	It is inferred from the text that the outcome was 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 (measurements were automated).
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Information on initial conditions for each study group are not reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Information on study group differences unrelated to test substance are not fully reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Sufficient data were provided to conduct independent statistical analysis (presented as means +/- SD of 8 treated cultures from two independent experiments).
	Metric 23:	Data Interpretation	Low	× 2	6	The criteria for a positive response were not explicitly specified. Based on information in the results, it can be inferred that the dose-relatedness of the response was considered.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cytotoxicity data were included in evaluation. The study indicated that cytotoxicity was measured as LDH release from cells; these data were presented alongside the UDS data.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported by exposure group (Figure 1).
Overall Quality Determination‡			Medium		1.7	

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Study Citation:	S. Vamvakas, W. Dekant, D. Henschler (1989). Genotoxicity of haloalkene and haloalkane glutathione S-conjugates in porcine kidney cells Toxicology In Vitro, 3(2,2), 151-156
Data Type:	UDS for perc metabolite (TCVG)
HERO ID:	629910

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.

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

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

Table 77: **In vitro evaluation results for Vamvakas et al 1989 for Ames test study**

Study Citation:	S. Vamvakas, M. Herkenhoff, W. Dekant, D. Henschler (1989). Mutagenicity of tetrachloroethene in the ames test: Metabolic activation by conjugation with glutathione Journal of Biochemical Toxicology, 4(1,1), 21-27
Data Type:	Ames test for perc
HERO ID:	629911

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as tetrachloroethene.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of tetrachlorothene was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	Tetrachloroethene, purchased from a commercial source, we further purified by distillation; purity was determined to be 99.9% as determined by GC-MS.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using a concurrent negative (solvent-only) control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control was not used; however, test substances used in the study elicited positive responses (indicating that the assay is capable of detecting a positive response).
	Metric 6:	Assay Procedures	High	× 1	1	Methods and procedures were described. It was indicated that the mutagenicity system applied was a modified pre-incubation method similar to that described by Maron and Ames (1983).
	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	Test substance preparation was reported (dissolved in DMSO). Storage was not reported (but is not expected to impact the study results).
	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	The dose of perc (2 mM) used in the Ames pre-incubation test including GSH S-transferase, GSH, and liver microsomes was reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The duration of pre-incubation was reported (0-180 minutes) and was appropriate. The study showed that perc incubated with purified GSH-S-transferase and GSH produced a time-dependent formation of TCVG.

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Study Citation:	S. Vamvakas, M. Herkenhoff, W. Dekant, D. Henschler (1989). Mutagenicity of tetrachloroethene in the ames test: Metabolic activation by conjugation with glutathione Journal of Biochemical Toxicology, 4(1,1), 21-27
Data Type:	Ames test for perc
HERO ID:	629911

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	In the study using perc incubated with GSH S-transferase, GSH, and liver microsomes, only one dose was used (over a course of pre-incubation times); the study indicated that experiments were conducted with TCVG (a metabolite) to determine the optimum conditions for perc (the parent compound).
	Metric 13:	Metabolic Activation	Low	× 1	3	Exposures were conducted in absence and presence of metabolic activators (e.g., male rat kidney or liver fractions). Details on activators were not reported.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Test models were reported with limited descriptive information. However, the strains (Salmonella typhimurium strains TA 100, TA 98, and/or TA 2638) were obtained from laboratory-maintained cultures, their properties were checked regularly, and the they are routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	In the study using perc incubated with GSH S-transferase, GSH, and liver microsomes, it was indicated that data points were for 4 determinations from 2 independent experiments.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	It appears that outcomes were assessed consistently across study groups (revertant colonies counted after 2 days incubation).
	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 (colony counting was automated).
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	Medium	× 1	2	Data on disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Data were presented as means +/- SD, and n was reported. Although statistical analyses could be conducted, they are not necessary by study type.

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Study Citation:	S. Vamvakas, M. Herkenhoff, W. Dekant, D. Henschler (1989). Mutagenicity of tetrachloroethene in the ames test: Metabolic activation by conjugation with glutathione Journal of Biochemical Toxicology, 4(1,1), 21-27
Data Type:	Ames test for perc
HERO ID:	629911

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 23:	Data Interpretation	Medium	× 2	4	Based on information contained in the results section, it can be inferred that a doubling in the frequency of revertant colonies was considered the criteria for a positive response.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity data were not reported (not strictly required by study type).
	Metric 25:	Reporting of Data	Medium	× 2	4	In the study using perc incubated with GSH S-transferase, GSH, and liver microsomes, data were presented for each time point. Data for mutagenicity tests of perc without activation or with activation (rat liver S9 or microsomes only) were not shown (doses not explicitly specified).
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 78: **In vitro evaluation results for Vamvakas et al 1989 for Ames test study**

Study Citation:	S. Vamvakas, M. Herkenhoff, W. Dekant, D. Henschler (1989). Mutagenicity of tetrachloroethene in the ames test: Metabolic activation by conjugation with glutathione Journal of Biochemical Toxicology, 4(1,1), 21-27
Data Type:	Ames for perc metabolite (TCVG)
HERO ID:	629911

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as S-(1,2,2-trichlorovinyl)glutathione (TCVG).
	Metric 2:	Test Substance Source	Medium	× 1	2	The test substance was synthesized and characterized as described in a previous publication.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was determined to be 99.5% pure based on HPLC/UV-detection at 220 m.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using a concurrent negative (solvent-only) control.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control was not used; however, test substances used in the study elicited positive responses (indicating that the assay is capable of detecting a positive response).
	Metric 6:	Assay Procedures	High	× 1	1	Methods and procedures were described. It was indicated that the mutagenicity system applied was a modified pre-incubation method similar to that described by Maron and Ames (1983).
	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	Test substance preparation was reported (dissolved in methanol). Storage was not reported (but is not expected to impact the study results).
	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	Doses for the assay conducted in Salmonella typhimurium strain TA 100 were reported without ambiguity (can be estimated from Figure 1).
	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	Medium	× 1	2	The number of dose groups was reported (at least 5 plus controls for studies using S. typhimurium TA 100). A rationale for dose selection was not provided.

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Study Citation:	S. Vamvakas, M. Herkenhoff, W. Dekant, D. Henschler (1989). Mutagenicity of tetrachloroethene in the ames test: Metabolic activation by conjugation with glutathione Journal of Biochemical Toxicology, 4(1,1), 21-27
Data Type:	Ames for perc metabolite (TCVG)
HERO ID:	629911

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Low	× 1	3	Exposures were conducted in absence and presence of metabolic activators (e.g., male rat kidney or liver fractions). Details on activators were not reported.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Test models were reported with limited descriptive information. However, the strains (Salmonella typhimurium strains TA 100, TA 98, and/or TA 2638) were obtained from laboratory-maintained cultures, their properties were checked regularly, and the they are routinely used for the outcome of interest.
	Metric 15:	Number per Group	High	× 1	1	Data points (Figure 1) were for 4 determinations from 2 independent experiments.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology was sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	It appears that outcomes were assessed consistently across study groups (revertant colonies counted after 2 days incubation).
	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 (colony counting was automated).
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	Medium	× 1	2	Data on disproportionate outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Data were presented as means +/- SD, and n was reported. Although statistical analyses could be conducted, they are not necessary by study type.
	Metric 23:	Data Interpretation	Medium	× 2	4	Based on information contained in the results section, it can be inferred that a doubling in the frequency of revertant colonies was considered the criteria for a positive response.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity data were not reported (not strictly required by study type).

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Study Citation:	S. Vamvakas, M. Herkenhoff, W. Dekant, D. Henschler (1989). Mutagenicity of tetrachloroethene in the ames test: Metabolic activation by conjugation with glutathione Journal of Biochemical Toxicology, 4(1,1), 21-27
Data Type:	Ames for perc metabolite (TCVG)
HERO ID:	629911

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	Data were reported for S.typhimurium strain TA 100 by exposure group. Data for S. typhmiurium strains TA 2638 and TA 98 and for experiments that varied in pre-incubation time were described qualitatively (negative).
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.

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

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

Table 79: **In vitro evaluation results for Wang et al 2001 for micronucleus assay study**

Study Citation:	J. L. Wang, W. L. Chen, S. Y. Tsai, P. Y. Sung, R. N. Huang (2001). An in vitro model for evaluation of vaporous toxicity of trichloroethylene and tetrachloroethylene to CHO-K1 cells Chemico-Biological Interactions, 137(2,2), 139-154
Data Type:	Micronucleus assay for perc
HERO ID:	629916

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name and CASRN.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported. Although a batch/lot number was not provided, the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The purity of the test substance was reported (99%); purity was such that effects were likely due to the test substance itself.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	The study authors reported using concurrent negative controls; the type of control used (untreated or solvent-only) was not clearly specified.
	Metric 5:	Positive Controls	Not Rated	NA	NA	A positive control is not strictly required by study type. Test substances used in the assay produced positive, dose-related responses (indicative that the assay was effective).
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were briefly described and cited to another publication (Fenech 1993).
	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	Storage was not reported (but not expected to impact the study results). The study indicated that the test substance was added as a liquid to a central (open) glass dish and allowed to evaporate and dissolve in the surrounding medium (closed, but not sealed petri dish containing cultured cells). Although there was evidence that the test substance volatilized from the test vessels, actual test substance concentrations (while extremely low) were measured by gas chromatography.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	It was inferred that exposures were administered consistently across study groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses (after 24 hours exposure) could be estimated from Figure 2.

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Study Citation: J. L. Wang, W. L. Chen, S. Y. Tsai, P. Y. Sung, R. N. Huang (2001). An in vitro model for evaluation of vaporous toxicity of trichloroethylene and tetrachloroethylene to CHO-K1 cells Chemico-Biological Interactions, 137(2,2), 139-154
Data Type: Micronucleus assay for perc
HERO ID: 629916

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Low	× 2	6	The exposure duration was reported (24 hours), but exceeded the recommendation for this study type.
	Metric 12:	Exposure Route and Method	Low	× 1	3	The number of dose groups was reported (3 plus controls) and appropriate. However, owing to the volatility of the test substance, actual test concentrations fell into a narrow (less than 2-fold) range. In addition, cytotoxicity was excessive (particularly at the two highest tested concentrations).
	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	Low	× 2	6	The test model was reported (CHO-K1 cells); this cell type is routinely used in genotoxicity tests. However, the test model was identified with little to no additional information (e.g., source).
	Metric 15:	Number per Group	High	× 1	1	The study indicated that results represented four independent experiments.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology appeared to be appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	The outcome assessment was inferred to be consistent across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	The study indicated that 500 binucleated cells per dish were examined (i.e., 2000 cells/dose group).
	Metric 19:	Blinding of Assessors	High	× 1	1	It was indicated that the dishes were blindly coded.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No confounding differences were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	No confounding variables unrelated to exposure were reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistics were reported and were appropriate for the study type and data presented. The data shown graphically (means +/-SD) are also sufficient for independent analyses.
	Metric 23:	Data Interpretation	Medium	× 2	4	While not explicitly specified, the statistical significance and dose-relatedness of the response appeared to be the criteria for a positive response.

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Study Citation:	J. L. Wang, W. L. Chen, S. Y. Tsai, P. Y. Sung, R. N. Huang (2001). An in vitro model for evaluation of vaporous toxicity of trichloroethylene and tetrachloroethylene to CHO-K1 cells Chemico-Biological Interactions, 137(2,2), 139-154
Data Type:	Micronucleus assay for perc
HERO ID:	629916

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Cytotoxicity Data	Medium	× 1	2	Cytotoxicity methods were briefly reported (i.e., cell count using a hematocytometer).
	Metric 25: Reporting of Data	High	× 2	2	Data was reported for each exposure group.
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 80: **In vitro evaluation results for White et al 2001 for human lymphoblastoma micronucleus study**

Study Citation:	I. N. White, N. Razvi, A. H. Gibbs, A. M. Davies, M. Manno, C. Zaccaro, F. De Matteis, A. Pahler, W. Dekant (2001). Neoantigen formation and clastogenic action of HCFC-123 and perchloroethylene in human MCL-5 cells Toxicology Letters, 124(1-3,1-3), 129-138
Data Type:	micronucleus assay in human lymphoblastoma cells
HERO ID:	631154

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified as perchloroethylene
	Metric 2:	Test Substance Source	High	× 1	1	The source of the test substance was reported (Sigma Chemical)
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity of test substance was not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Study authors report using a vehicle control.
	Metric 5:	Positive Controls	High	× 2	2	Tamoxifen was used as a positive control and the response was appropriate (mean and standard deviation of positive control was described in text).
	Metric 6:	Assay Procedures	High	× 1	1	Study authors described the methods and procedures used for the test and they were applicable for the study type.
	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	High	× 1	1	Test substance preparation was described as dissolved in DMSO; storage was not reported but this is appropriate given the study design (single-dose administration).
	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 (Table 2).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate (24 hr). Typically only a 3-6 hr exposure is necessary for the in vitro micronucleus assay, but longer exposures are acceptable.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure concentrations were reported; the number of groups and spacing was not justified by the study authors, but the number of exposure groups and spacing of exposure levels were adequate to show results relevant to the outcome of interest.

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Study Citation:	I. N. White, N. Razvi, A. H. Gibbs, A. M. Davies, M. Manno, C. Zaccaro, F. De Matteis, A. Pahler, W. Dekant (2001). Neoantigen formation and clastogenic action of HCFC-123 and perchloroethylene in human MCL-5 cells Toxicology Letters, 124(1-3,1-3), 129-138
Data Type:	micronucleus assay in human lymphoblastoma cells
HERO ID:	631154

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Exposures were conducted in MCL-5 cells that express a high level of native CYP1A1 and transfected CYP1A2, CYP2E1, CYP2A6, and CYP3A4.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test model was reported with limited descriptive information. The cells were obtained from a commercial source (Gentest Corp). This cell line is not routinely used for this assay; however, is appropriate for the outcome of interest
	Metric 15:	Number per Group	High	× 1	1	4 replicates per treatment group were included in the study design.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were reported and 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	Low	× 2	6	250 binucleated cells were scored per replicate (total of 1000 cells per treatment group). This is considered lacking with respect to current standards and guidelines (2000 binucleated cells per treatment group).
	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	High	× 2	2	No differences in initial quality of tissues exposed or lot of test substance were reported.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was conducted; dose response was determined using regression analysis. Independent statistical analysis to compare individual treatment group responses may be conducted, as sufficient summary data are provided (mean, standard deviation, and sample size).
	Metric 23:	Data Interpretation	Medium	× 2	4	Clastogenicity was considered an effect if the number of micronuclei compared to the control was significantly different. Criteria for a positive result were possibly provided in the cited reference for the micronucleus assay procedures (White et al. 1992).

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Study Citation:	I. N. White, N. Razvi, A. H. Gibbs, A. M. Davies, M. Manno, C. Zaccaro, F. De Matteis, A. Pahler, W. Dekant (2001). Neoantigen formation and clastogenic action of HCFC-123 and perchloroethylene in human MCL-5 cells Toxicology Letters, 124(1-3,1-3), 129-138
Data Type:	micronucleus assay in human lymphoblastoma cells
HERO ID:	631154

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were not described. However, a clear dose-response relationship was established, demonstrating a range of responses (i.e. all doses were not too high to induce toxicity at all doses). Given the positive results, this is considered acceptable.
	Metric 25: Reporting of Data	High	× 2	2	Data for the outcomes were presented for each exposure group as a mean and SD in Table 2.
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 81: **In vitro evaluation results for NTP 1986 for mutagenicity study**

Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	PERC mouse Lymphoma TK mutagenicity
HERO ID:	632655

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Reported by name CAS, structure, and MW (assumed same as in vivo studies)
	Metric 2:	Test Substance Source	High	× 1	1	Assumed to be the same as the in vivo studies: Dow Chemical, lot TA03116F-01; purity and identity analyses conducted
	Metric 3:	Test Substance Purity	High	× 1	1	Assumed to be the same as the rat and mouse studies (confirmed analytically - approximately 99.9%)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative (solvent) control was reported
	Metric 5:	Positive Controls	High	× 2	2	Positive control were reported and appeared to be appropriate,
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay procedures were cited to another publication with limited details reported
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Perc was found to be stable for 2 weeks at 60" C (Appendix H). Perc was prepared in DMSO and added to cell media.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure is assumed to be consistent across all groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported clearly in the tables
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appropriate for the study type; 4h wash then 48h exposure
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Number of exposure groups was reported and appropriate, spacing was not justified but appeared appropriate
	Metric 13:	Metabolic Activation	High	× 1	1	S9 is produced from aroclor 1254 induced male SD rats and syrian hamster
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Test model was previously cited along with limited description but is commonly used
	Metric 15:	Number per Group	High	× 1	1	Cell number was cited in TK and appropriate, replicates were reported duplicate or triplicate for TK

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Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	PERC mouse Lymphoma TK mutagenicity
HERO ID:	632655

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	Not Rated	NA	NA	Study methods were cited to another publication (Clive et al 1979)
	Metric 17: Consistency of Outcome Assessment	Not Rated	NA	NA	Study methods were cited to another publication
	Metric 18: Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study type
	Metric 19: Blinding of Assessors	Not Rated	NA	NA	Blinding was 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 attrition and/or health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis					
	Metric 22: Data Analysis	High	× 1	1	Statistics were not reported , but results were reported sufficiently for independent analysis.
	Metric 23: Data Interpretation	Low	× 2	6	Scoring and evaluation criteria were not reported.
	Metric 24: Cytotoxicity Data	Low	× 1	3	Cytotoxicity data and endpoint were not defined
	Metric 25: Reporting of Data	Medium	× 2	4	Data were reported for all groups and outcomes; footnote for Table G6 indicate that data from only one experiment was shown (not mean of replicates).
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 82: **In vitro evaluation results for NTP 1986 for bacterial mutagenicity study**

Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	PERC bacterial mutagenicity
HERO ID:	632655

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Reported by name CAS, structure, and MW (assumed same as in vivo studies)
	Metric 2:	Test Substance Source	High	× 1	1	Assumed to be the same as the in vivo studies: Dow Chemical, lot TA03116F-01; purity and identity analyses conducted
	Metric 3:	Test Substance Purity	High	× 1	1	Assumed to be the same as the rat and mouse studies (confirmed analytically - approximately 99.9%)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative (solvent) control was reported
	Metric 5:	Positive Controls	Not Rated	NA	NA	No positive control was reported
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay procedures were cited to another publication with limited details reported
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Perc was found to be stable for 2 weeks at 60" C (Appendix H). Perc was prepared in DMSO and added to cell media
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure is assumed to be consistent across all groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported clearly in the tables
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appropriate for the study type; 48h exposure
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Number of exposure groups was reported and appropriate, spacing was not justified but appeared appropriate
	Metric 13:	Metabolic Activation	High	× 1	1	S9 is produced from Aroclor 1254 induced male SD rats and syrian hamster
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	Test model was previously cited along with limited description but is commonly used
	Metric 15:	Number per Group	High	× 1	1	Number may have been previously cited, replicates were reported triplicate
Domain 5: Outcome Assessment						

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Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	PERC bacterial mutagenicity
HERO ID:	632655

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Study methods were cited to another publication (Haworth et al 1983)
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Study methods were cited to another publication
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study type
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was 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 attrition and/or health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistics were not reported , but results were reported sufficiently for independent analysis
	Metric 23:	Data Interpretation	Low	× 2	6	Scoring and evaluation criteria were not reported
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity data was reported in the ames table but the endpoint was not defined.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all groups and outcomes
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 83: **In vitro evaluation results for NTP 1986 for sister chromatid exchange study**

Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)					
Data Type:	PERC SCE and CAs in CHO					
HERO ID:	632655					
Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Reported by name CAS, structure, and MW (assumed same as in vivo studies)
	Metric 2:	Test Substance Source	High	× 1	1	Assumed to be the same as the in vivo studies: Dow Chemical, lot TA03116F-01; purity and identity analyses conducted
	Metric 3:	Test Substance Purity	High	× 1	1	Assumed to be the same as the rat and mouse studies (confirmed analytically - approximately 99.9%)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Negative (solvent) control was reported
	Metric 5:	Positive Controls	High	× 2	2	Positive control were reported and appeared to be appropriate: -/+ S9 triethanolamine/cyclophosphamide
	Metric 6:	Assay Procedures	Not Rated	NA	NA	Assay procedures were cited to another publication with limited details reported
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable to the study type
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	Perc was found to be stable for 2 weeks at 60" C (Appendix H). Perc was prepared in DMSO and added to cell media
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure is assumed to be consistent across all groups
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported clearly in the tables
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Exposure duration was reported and appropriate for the study type. SCE: 2 hours with Perc then, 24h (-S9) or 26 h (+S9) with Perc and Brdu, then 2-3 h with colcemid . CA 8-10 h plus 2-3h with colcemid
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Number of exposure groups was reported 3-4 doses plus controls . Appropriate spacing was not justified but appeared appropriate, though it is unclear whether the high dose was sufficient.
	Metric 13:	Metabolic Activation	High	× 1	1	S9 is produced from Aroclor 1254 induced male SD rats and syrian hamster
Domain 4: Test Model						

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Study Citation:	NTP (1986). Toxicology and carcinogenesis studies of tetrachloroethylene (perchloroethylene) (CAS no. 127-18-4) in F344/N rats and B6C3F1 mice (inhalation studies)
Data Type:	PERC SCE and CAs in CHO
HERO ID:	632655

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	Test model was previously cited along with limited description but is commonly used
	Metric 15:	Number per Group	Not Rated	NA	NA	Number and replicate may have been previously cited.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Not Rated	NA	NA	Study methods were cited to other publications.
	Metric 17:	Consistency of Outcome Assessment	Not Rated	NA	NA	Study methods were cited to other publications.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Based on staining (Giemsa, previously cited), sampling adequacy is inferred to be autocounted. Study methods were cited to other publications.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding was not reported, but is assumed to be not applicable due to giemsa staining indicating auto count
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 attrition and/or health outcomes unrelated to exposure for each study group were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistics were not reported , and no replicates were reported, however, results were clearly negative across groups
	Metric 23:	Data Interpretation	Low	× 2	6	Scoring and evaluation criteria were not reported.
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity data and endpoint were not defined
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported for all groups and outcomes
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 84: **In vitro evaluation results for Shimada et al 1985 for bacterial reverse mutation study**

Study Citation:	T. Shimada, A. F. Swanson, P. Leber, G. M. Williams (1985). Activities of chlorinated ethane and ethylene compounds in the Salmonella/rat microsome mutagenesis and rat hepatocyte/DNA repair assays under vapor phase exposure conditions Cell Biology and Toxicology, 1(3,3), 159-179
Data Type:	Bacterial reverse mutation for perc
HERO ID:	632848

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by established nomenclature.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified. A batch/lot number was not given, but the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be >99% pure (99.99% for high-purity, 99.93% for low-stabilized, and 99.80% for stabilized forms).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using non-exposed controls.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were used (vinyl chloride) and responded appropriately.
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and also cited in other publications, but appeared to be appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage were well-described and appropriate for the test substance.
	Metric 9:	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 10:	Reporting of Doses/Concentrations	High	× 2	2	Vapor concentrations were reported.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration for the bacterial mutation assay was reported to be 18h with a total incubation time of 48-72h.

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Study Citation:	T. Shimada, A. F. Swanson, P. Leber, G. M. Williams (1985). Activities of chlorinated ethane and ethylene compounds in the Salmonella/rat microsome mutagenesis and rat hepatocyte/DNA repair assays under vapor phase exposure conditions Cell Biology and Toxicology, 1(3,3), 159-179
Data Type:	Bacterial reverse mutation for perc
HERO ID:	632848

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	Cytotoxicity data were used to justify exposure analyzable concentrations. The number of exposure groups was not explicitly specified (2 or 3 doses were shown in Table 6). A range of doses from 1.0% (stabilized) or 2.5% (low-stabilized) to 10% was reported in the legend for Table 6, with some doses not shown in the table owing to total cell death.
	Metric 13:	Metabolic Activation	Medium	× 1	2	The presence of a commonly used metabolic activation system was reported (S9 from Aroclor 1254 indcued rats); however, some details regarding type, composition mix, concentration, or quality control information were not described.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test models were reported along with limited descriptive information and were routinely used for the outcomes of interest.
	Metric 15:	Number per Group	High	× 1	1	There were 3 replicates for each experiment. Based on Table 6, it appears that 2 experiments were conducted under experimental conditions (presumably all doses/forms/strains) and 8 experiments for controls (i.e., spontaneous revertants).
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methods addressed and were sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment were reported and were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcomes of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences reported among initial study group parameters.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	There were no reported differences among the study replicates or groups in test models unrelated to exposure.
Domain 7: Data Presentation and Analysis						

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Study Citation:	T. Shimada, A. F. Swanson, P. Leber, G. M. Williams (1985). Activities of chlorinated ethane and ethylene compounds in the Salmonella/rat microsome mutagenesis and rat hepatocyte/DNA repair assays under vapor phase exposure conditions Cell Biology and Toxicology, 1(3,3), 159-179
Data Type:	Bacterial reverse mutation for perc
HERO ID:	632848

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analyses were not performed, but may not be strictly required.
	Metric 23:	Data Interpretation	High	× 2	2	Study authors reported the evaluation criteria for determining a positive outcome which were consistent with established practices (more than 2-fold increase over controls).
	Metric 24:	Cytotoxicity Data	High	× 1	1	The methods for measuring cytotoxicity were clearly described and commonly used for assessment.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were provided by exposure group (Table 6).
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 85: **In vitro evaluation results for Shimada et al 1985 for DNA repair study in rat hepatocytes**

Study Citation:	T. Shimada, A. F. Swanson, P. Leber, G. M. Williams (1985). Activities of chlorinated ethane and ethylene compounds in the Salmonella/rat microsome mutagenesis and rat hepatocyte/DNA repair assays under vapor phase exposure conditions Cell Biology and Toxicology, 1(3,3), 159-179
Data Type:	DNA repair in rat hepatocytes for perc
HERO ID:	632848

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by established nomenclature.
	Metric 2:	Test Substance Source	High	× 1	1	The manufacturer was identified. A batch/lot number was not given, but the test substance is not expected to vary in composition.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was >99% pure (99.99% for the high purity, 99.93% for low-stabilized, and 99.80% for stabilized forms).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	The study authors reported using non-exposed controls. Fluorene was also used as a negative control in the conventional (liquid) assay.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were used (2-acetyl amino fluorene for liquid assay; monochloroethylene for vapor exposure).
	Metric 6:	Assay Procedures	Medium	× 1	2	Methods and procedures were partially described and also cited in other publications, but appeared to be appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage were well-described and appropriate for the test substance.
	Metric 9:	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 10:	Reporting of Doses/Concentrations	High	× 2	2	Vapor and liquid concentrations were reported (as %).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration was reported to be 3 hours or 18 hours. The study provided a rationale for the duration of exposure (e.g., based on a preliminary dose-finding study using monochloroethylene). However, reducing the duration of exposure to 3 hours did not reduce cytotoxicity.

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Study Citation:	T. Shimada, A. F. Swanson, P. Leber, G. M. Williams (1985). Activities of chlorinated ethane and ethylene compounds in the Salmonella/rat microsome mutagenesis and rat hepatocyte/DNA repair assays under vapor phase exposure conditions Cell Biology and Toxicology, 1(3,3), 159-179
Data Type:	DNA repair in rat hepatocytes for perc
HERO ID:	632848

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Low	× 1	3	Doses were based on a preliminary dose-finding study. However, test substances used in the assay were more cytotoxic than monochloroethylene (used in the preliminary assay). In the vapor assay with 3 or 18 hours exposure, there was complete toxicity at the two highest doses for all forms of perc (leaving only one analyzable dose). In the conventional (liquid) assay with 18 hours exposure, there was complete toxicity at the highest dose for the low-stabilized form and nearly complete toxicity at the highest dose for the stabilized form. After 3 hours exposure, there was complete toxicity at the highest dose for the low-stabilized form and at the highest dose for the stabilized form (leaving only one analyzable dose).
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Exogenous metabolic activation was not needed for rat hepatocytes.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The test models were reported along with limited descriptive information and were routinely used for the outcomes of interest.
	Metric 15:	Number per Group	High	× 1	1	The study indicated that 3 replicates were used.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methods addressed and were sensitive for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Details of the outcome assessment were reported and were assessed consistently across study groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to the outcomes of interest.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcomes of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences reported among initial study group parameters.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	There were no reported differences among the study replicates or groups in test models unrelated to exposure.
Domain 7: Data Presentation and Analysis						

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Study Citation:	T. Shimada, A. F. Swanson, P. Leber, G. M. Williams (1985). Activities of chlorinated ethane and ethylene compounds in the Salmonella/rat microsome mutagenesis and rat hepatocyte/DNA repair assays under vapor phase exposure conditions Cell Biology and Toxicology, 1(3,3), 159-179
Data Type:	DNA repair in rat hepatocytes for perc
HERO ID:	632848

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 22:	Data Analysis	Not Rated	NA	NA	Statistical analyses were not performed, but may not be strictly required. Data provided would be amenable to statistical analyses.
	Metric 23:	Data Interpretation	Low	× 2	6	The study indicated that the criteria for a positive response was when the minimum net grain count exceeded 5 nuclei and was "significantly" above controls in 2 experiments. The rationale for this cut-off and the criteria for a significant response (in the absence of statistical analyses) was not clearly specified.
	Metric 24:	Cytotoxicity Data	High	× 1	1	The methods for measuring cytotoxicity were clearly described and commonly used for assessment.
	Metric 25:	Reporting of Data	High	× 2	2	Data for exposure-related findings were presented for all outcomes 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 86: **In vitro evaluation results of Beland 1999 study on bacterial reverse mutation**

Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Bacterial reverse mutation for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was clearly identified as chloral hydrate. In the NTP report, a CASRN, structure, and chemical formula were provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source of the test substance was reported (including lot number).
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance was reported to be 99% pure.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Appropriate concurrent negative (solvent-only) control groups were included.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls were tested concurrently with each test substance. The identity of each positive control was reported and appropriate for different strains with and without metabolic activation. Positive controls yielded positive results.
	Metric 6:	Assay Procedures	High	× 1	1	Assay methods and procedures were described in detail and were applicable to the study type. This evaluation form was completed with respect to Haworth et al. 1983 (HERO ID 28947), which was cited in Table E1 of Beland 1999 to contain the detailed protocol for the bacterial reverse mutation 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	High	× 1	1	Test substance preparation was reported. Test substance storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Exposure administration was consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration for the pre-incubation protocol was reported and appropriate.
	Metric 12:	Exposure Route and Method	High	× 1	1	The maximum dose was chosen based on solubility limits or cytotoxicity. The number of exposure groups was reported (at least 5 plus controls) and spacing was appropriate (100, 333, 1000, 3333, 4000, 5000, 6667, 7500, and/or 10000 µg/plate).

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Bacterial reverse mutation for chloral hydrate
HERO ID:	701161

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	High	× 1	1	The source and method of preparation of the rat liver S9 fraction was reported; the concentration of S9 in the bacterial mutagenicity assay was specified in the data table (10%).
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The identity and donor source of the bacterial strains used here were identified, and these strains are routinely used for the outcome of interest. It was noted in Haworth et al. (1983) that the cultures were "routinely checked for genetic integrity as recommended by Ames et al. (1975)."
	Metric 15:	Number per Group	High	× 1	1	Each assay was plated in triplicate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	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	No differences among treatment group parameters were reported.
	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	Not Rated	NA	NA	Statistical analysis not required by study type. However, raw data were provided and could be analyzed independently.
	Metric 23:	Data Interpretation	High	× 2	2	The criteria for a positive (as well and negative and equivocal) response were reported. A response was considered positive if a reproducible, dose-related increase in revertant colonies was observed (no minimum fold-increase required).
	Metric 24:	Cytotoxicity Data	High	× 1	1	According to Haworth et al. (1983), a dose-setting experiment was conducted to assess cytotoxicity (viability based on reduced numbers of colonies). Doses for the mutagenicity assay were selected so that the highest dose exhibited some degree of toxicity.

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Bacterial reverse mutation for chloral hydrate
HERO ID:	701161

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	All data are adequately reported.
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 87: **In vitro evaluation results of Beland 1999 study on bacterial reverse mutation**

Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Bacterial reverse mutation for TCE metabolites
HERO ID:	701161

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TCE metabolites were clearly identified by name (chloral hydrate, trichloroacetic acid, trichloroethanol).
	Metric 2:	Test Substance Source	Low	× 1	3	The commercial source of the test substances was not 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	Low	× 2	6	Negative controls were included based on Figure D12, but further details were not provided.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not reported to be included in the study design. However, positive results were obtained; therefore, this demonstrates the ability of the lab to detect a positive result.
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay methods and procedures were briefly described and cited to other references (Maron and Ames 1983), but appeared appropriate.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	This metric is not applicable to this study type.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Unacceptable	× 1	4	Test substance preparation and/or vehicle was not reported. Storage was not reported (but not expected to impact the study results).
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Exposure administration was inferred to be consistent across treatment groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Doses were reported (and can be estimated from Figure D12).
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration for the pre-incubation protocol was reported and appropriate. The exposure duration for the direct plate incorporation method was not reported, but assumed to be appropriate considering the citation for the protocol (Maron and Ames 1983).
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The number of exposure groups was reported (at least 4 plus controls) and appropriate for this assay.

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Bacterial reverse mutation for TCE metabolites
HERO ID:	701161

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Medium	× 1	2	The source and method of preparation of the rat liver S9 fraction was reported; however, the concentration of S9 in the bacterial mutagenicity assay was not specified (assumed to be appropriate based on cited publication).
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The identity of the S. typhimurium strain TA 104 was identified. No further details were provided. This strain is routinely used for the outcome of interest.
	Metric 15:	Number per Group	Low	× 1	3	The number of plates per treatment group was not reported. It is likely that one plate per treatment group was utilized, as there are no error bars on the graph in Figure D12. This is considered acceptable for the bacterial reverse mutation assay.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology is appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	The outcome assessment was consistent across treatment groups.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	This metric is not applicable to this endpoint.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	Low	× 2	6	No differences among treatment group parameters were reported.
	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	Not Rated	NA	NA	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	Evaluation criteria were not explicitly specified.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	It is not apparent that cytotoxicity was assessed or considered in the study design or interpretation of results (but not strictly required by study type).

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Study Citation:	F. Beland (1999). NTP technical report on the toxicity and metabolism studies of chloral hydrate (CAS No. 302-17-0). Administered by gavage to F344/N rats and B6C3F1 mice Toxicity Report Series, 59(59,59), 1-66, A1-E7
Data Type:	Bacterial reverse mutation for TCE metabolites
HERO ID:	701161

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 25: Reporting of Data	High	× 2	2	Data were reported by exposure group (Figure D12).
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 =≥ 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 88: **In vitro evaluation results for Benane et al 1996 for intracellular communication study**

Study Citation:	S. Benane, C. Blackman, D. House (1996). Effect of perchloroethylene and its metabolites on intercellular communication in clone 9 rat liver cells Journal of Toxicology and Environmental Health, 48(5,5), 427-437
Data Type:	Intercellular communication- Perc , DCA, TCA, CH, and TCOH
HERO ID:	701166

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substances were identified as perchloroethylene (Perc), dichloroacetic acid (DCA), trichloroacetic acid (TCA), chloral hydrate (CH), 2,2,2-trichloroethanol (TCEth)
	Metric 2:	Test Substance Source	High	× 1	1	All five test substances were obtained from Sigma Chemical Corp., St Louis, MO)
	Metric 3:	Test Substance Purity	Low	× 1	3	The purity or grade of test substances were not reported.
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	A concurrent negative control was included (0 mM test substance), however it is unclear if the negative control contained the vehicle (acetone for Perc; water for all other test substances).
	Metric 5:	Positive Controls	High	× 2	2	Positive control TPA was run as a calibration chemical and to define the response.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedure and method were fully described.
	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	High	× 1	1	Preparation of test substances were well-described. Test substance storage was not reported, but this is appropriate given the study design (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Details of exposure administration were limited; however this is unlikely to have a substantial impact on results.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Dose concentrations are reported without ambiguity.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported and appropriate for this study.
	Metric 12:	Exposure Route and Method	High	× 1	1	Dose concentration spacing was adequate to show a dose-response.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not necessary since Clone 9, a normal liver epithelial cell line was used.
Domain 4: Test Model						

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Study Citation:	S. Benane, C. Blackman, D. House (1996). Effect of perchloroethylene and its metabolites on intercellular communication in clone 9 rat liver cells Journal of Toxicology and Environmental Health, 48(5,5), 427-437
Data Type:	Intercellular communication- Perc , DCA, TCA, CH, and TCOH
HERO ID:	701166

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	Medium	× 2	4	Test model was reported as Clone 9, a normal liver epithelial cell line. However, limited information on the cells was included.
	Metric 15:	Number per Group	High	× 1	1	Each concentration was run in quadruplet.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome methodology were partially reported and cited elsewhere.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	There were incomplete reporting of details of outcome assessment protocol, however this is unlikely to have a substantial impact
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for this study.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No differences were reported in initial conditions for each study group.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Data on disproportionate outcomes unrelated to exposure were not reported, this is unlikely to have a substantial impact on results.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Appropriate statistical analysis was performed on the data. Independent statistical analysis could be conducted by estimating mean and standard error from the graphs.
	Metric 23:	Data Interpretation	High	× 2	2	Scoring and evaluation were appropriate.
	Metric 24:	Cytotoxicity Data	High	× 1	1	Cell viability was assessed with trypan blue.
	Metric 25:	Reporting of Data	High	× 2	2	Exposure related data were presented for all 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 89: **In vitro evaluation results for Matsushima et al 1999 for micronucleus study**

Study Citation:	T. Matsushima, M. Hayashi, A. Matsuoka, M. Ishidate, K. F. Miura, H. Shimizu, Y. Suzuki, K. Morimoto, H. Ogura, K. Mure, K. Koshi, T. Sofuni (1999). Validation study of the in vitro micronucleus test in a Chinese hamster lung cell line (CHL/IU) Mutagenesis, 14(6,6), 569-580
Data Type:	MN and CA assay
HERO ID:	716645

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test material identified as tetrachloroethylene, CASRN was provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source (Sigma) was 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	Solvent (DMSO) controls were used.
	Metric 5:	Positive Controls	High	× 2	2	A dedicated positive control was not included, and the results for the test substance were negative, however, 66 chemicals were tested overall, and more than half were clearly positive for MN demonstrating the validity of the test. Mitomycin C and methyl methanesulfonate were included and are standard positive controls for the CA and MN assays without metabolic activation and yielded positive responses.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described in sufficient detail, and were appropriate for the outcome of interest.
	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	The test substance was dissolved in solvent immediately prior to treatment. Storage conditions were not reported, but this is appropriate given the study design (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Consistency across groups was inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The dose range was reported. Specific doses can be determine from the figure provided.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	A number of exposure durations (options) were reported in the methods. These durations are appropriate for the outcomes of interest. Based on the available data presented it is assumed that only certain conditions/durations were evaluated for each test chemical.

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Study Citation: T. Matsushima, M. Hayashi, A. Matsuoka, M. Ishidate, K. F. Miura, H. Shimizu, Y. Suzuki, K. Morimoto, H. Ogura, K. Mure, K. Koshi, T. Sofuni (1999). Validation study of the in vitro micronucleus test in a Chinese hamster lung cell line (CHL/IU) Mutagenesis, 14(6,6), 569-580
Data Type: MN and CA assay
HERO ID: 716645

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Unacceptable	× 1	4	Justification for the dose range tested was not provided. Results were negative and no cytotoxicity was measured/reported, so it is unclear whether the doses were adequate for testing the outcome of interest. The number of dose groups varied depending on treatment. In some cases only 2 dose-groups in addition to the negative controls were used, which is less than recommended by current standards.
	Metric 13:	Metabolic Activation	Medium	× 1	2	Metabolic activation was not included for this test substance and no justification was provided, despite the fact that other test substances in this report were tested with and without metabolic activation. The responses in the absence of activation were negative, and testing with metabolic activation would have been appropriate. However, it is not expected that this deficiency affected the validity of the results without metabolic activation.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	Test model (CHL/IU cells) and the commercial source was reported. The cells are routinely used for the outcome of interest.
	Metric 15:	Number per Group	Low	× 1	3	The study does not report the number of replicates. The figure legend indicates "1st and 2nd" for one condition (-S9, 72hrs), it is presumed this represents a duplicate of that condition. Other conditions were represented only once.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodologies were described appropriate for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistency of outcome assessment across groups is inferred from the text.
	Metric 18:	Sampling Adequacy	Low	× 2	6	The number of intact interphase cells (1000) scored is appropriate when duplicate cultures are used, but for conditions where a single test was done, this is less than the recommended 2,000 total.
	Metric 19:	Blinding of Assessors	High	× 1	1	All slides were coded and analyzed blind.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	No confounding variables in test design and procedure were reported.

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Study Citation:	T. Matsushima, M. Hayashi, A. Matsuoka, M. Ishidate, K. F. Miura, H. Shimizu, Y. Suzuki, K. Morimoto, H. Ogura, K. Mure, K. Koshi, T. Sofuni (1999). Validation study of the in vitro micronucleus test in a Chinese hamster lung cell line (CHL/IU) Mutagenesis, 14(6,6), 569-580
Data Type:	MN and CA assay
HERO ID:	716645

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Confounding variables in outcomes unrelated to exposures were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	Low	× 1	3	Statistical analysis were described in the methods (Fisher's Exact test for comparisons to negative controls) and Cochran Armitage trend test, however, the data presented does not indicate Means, and no measures of variance are provided, suggesting the data presented are from a single replicate. Statistical results are not included in the presented data. More clarity in the descriptions are needed.
	Metric 23:	Data Interpretation	High	× 2	2	The scoring/criteria used to identify a positive result is based on statistical significance., which is generally acceptable; descriptions defining final decisions of positive, weak, or negative responses were discussed.
	Metric 24:	Cytotoxicity Data	Not Rated	NA	NA	Cytotoxicity was not evaluated.
	Metric 25:	Reporting of Data	Medium	× 2	4	Data results are provided for three test conditions. Based on the information provided, it is not entirely clear whether these were the only conditions tested for this test substance, (e.g., metabolic activation and additional exposure durations were described in the methods, but it is not known whether these were intended/were tested with all of the chemicals evaluated). Chromosome Aberration data were qualitatively reported as negative.
Overall Quality Determination‡			Unacceptable⋆⋆		1.5	
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 90: **In vitro evaluation results for Emmert et al 2006 for Ames test study**

Study Citation:	B. Emmert, J. Bünger, K. Keuch, M. Müller, S. Emmert, E. Hallier, G. A. Westphal (2006). Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S. typhimurium strain YG7108pin3ERb5 Toxicology, 228(1,1), 66-76
Data Type:	Ames assay for Perc
HERO ID:	1006124

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance was identified by name as tetrachloroethylene; the CASRN was provided.
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source (Sigma-Aldrich) was reported.
	Metric 3:	Test Substance Purity	High	× 1	1	The test substance purity (=99.5%) was reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Use of concurrent solvent controls was reported
	Metric 5:	Positive Controls	High	× 2	2	A positive control (N-nitrosodiethylamine) was reported and gave expected results
	Metric 6:	Assay Procedures	Medium	× 1	2	Assay procedures were cited to a published study, and partially described
	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	Low	× 1	3	Slight discrepancies were identified in test substance solution preparation. The methods indicate solutions were prepared in DMSO, however the figure legend indicates the test substance was in ethanol. Test substance storage was not reported.
	Metric 9:	Consistency of Exposure Administration	Not Rated	NA	NA	Details of exposure methods were cited to another publication
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Initial tests with concentrations up to toxic concentrations, 5 mg/plate, or the solubility limit were performed. Specific concentrations in the final test are reported graphically and may be determined from the figures presented, however determining the specific concentrations may be difficult (crowded/overlapping means at lower concentrations)
	Metric 11:	Number of Exposure Groups and Concentration Spacing	Medium	× 2	4	The exposure duration for one strain was extended to 72 hrs to account for potential growth delay induced by some compounds.
	Metric 12:	Exposure Route and Method	Low	× 1	3	Based on the figures presented at least 7 concentrations were tested however, significant toxicity at the four high concentrations was reported so it is unclear if the concentrations tested were appropriate for the evaluating the outcome of interest.

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Study Citation: B. Emmert, J. Bünger, K. Keuch, M. Müller, S. Emmert, E. Hallier, G. A. Westphal (2006). Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S. typhimurium strain YG7108pin3ERb5 Toxicology, 228(1,1), 66-76
Data Type: Ames assay for Perc
HERO ID: 1006124

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Medium	× 1	2	Metabolic activation was required for the parent strain and was performed as described in another study, although use of phenobarbital/beta-naphthoflavone-induced S9 was reported.
Domain 4: Test Model						
	Metric 14:	Test Model	Medium	× 2	4	The study used S. typhimurium strain YG7108 (a methyltransferase deficient parent strain) and YG108pin3ERb5, which is a metabolically competent strain. These are non-standard strains for an AMES assay, but were used because they are reported to be more sensitive than normal strains.
	Metric 15:	Number per Group	Medium	× 1	2	The number of strains was lower than the typical number used for this study type however, with the strains used, 3-5 independent experiments were performed.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment method was reported (automated culture counting of revertant colonies) and appropriate
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcome assessment was performed consistently across groups
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study design.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study design. Colony counting was automated.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical analysis was not conducted, but means and standard deviations are represented in the figures.
	Metric 23:	Data Interpretation	High	× 2	2	Acceptance criteria for a positive test were reported ("solvent and positive controls within the historical range of our laboratory and an at least 2-fold elevated base rate with a dose-dependency for at least two consecutive concentrations").

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Study Citation:	B. Emmert, J. Bünger, K. Keuch, M. Müller, S. Emmert, E. Hallier, G. A. Westphal (2006). Mutagenicity of cytochrome P450 2E1 substrates in the Ames test with the metabolic competent S. typhimurium strain YG7108pin3ERb5 Toxicology, 228(1,1), 66-76
Data Type:	Ames assay for Perc
HERO ID:	1006124

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Cytotoxicity endpoints were partially defined (induction of microcolonies), but the methods of measurements were not fully described or reported.
	Metric 25:	Reporting of Data		× 2	NA	Results from the parent strain (with and without metabolic activation were not reported. The data presented in the figure lacks clarity (the figure legend indicates it is showing microcolonies, but the graph is labeled as revertants). The text makes a distinction between the two. Based on the information provided, it is unclear if the test substance induced only microcolonies (indicating toxicity), or if revertant colonies were also observed (indicating mutagenicity). The text reports the test substance was negative in the Ames test, but the data does not clearly indicate these results.
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 91: **In vitro evaluation results for Irving and Elfarra 2013 for Ames test study**

Study Citation:	Irving, R.,Elfarra, A. A. (2013). Mutagenicity of the cysteine S-conjugate sulfoxides of trichloroethylene and tetrachloroethylene in the Ames test Toxicology, 306 157-161
Data Type:	Ames assay for PERC metabolites TCVC and TCVCS
HERO ID:	2128042

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by name as the PERC metabolites S-(1,2,2-trichlorovinyl)-l-cysteine (TCVC), and S-(1,2,2-trichlorovinyl)-l-cysteine sulfoxide (TCVCS)
	Metric 2:	Test Substance Source	High	× 1	1	The metabolites were synthesized for the experiment and analytically verified by HPLC
	Metric 3:	Test Substance Purity	High	× 1	1	Purity was reported (>95%)
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	A negative (buffer) control was used.
	Metric 5:	Positive Controls	Low	× 2	6	A positive control (Sodium azide) was included, however results were not reported.
	Metric 6:	Assay Procedures	High	× 1	1	The assays and procedures relating to exposure were described in detail.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study design.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Medium	× 1	2	The test chemical was dissolved in buffer and added to the solution. Information on test chemical storage was not reported. For a short-term study this is not expected to significantly influence the results.
	Metric 9:	Consistency of Exposure Administration	Medium	× 1	2	Consistent administration across test groups is inferred from the text.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	A concentration range was reported, and specific concentrations can be determined from the dose-response curves provided.
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure duration was reported (20 min pre-incubation followed by 48hrs on a plate) and appropriate for the outcome of interest
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (5 to 13 depending on the metabolite tested) and spacing were reported and appropriate for the outcomes of interest.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not included (Perc metabolites tested directly)
Domain 4: Test Model						

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Study Citation:	Irving, R.,Elfarra, A. A. (2013). Mutagenicity of the cysteine S-conjugate sulfoxides of trichloroethylene and tetrachloroethylene in the Ames test Toxicology, 306 157-161
Data Type:	Ames assay for PERC metabolites TCVC and TCVCS
HERO ID:	2128042

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Test Model	High	× 2	2	The test model (S. typhimurium strain TA100) is appropriate and routinely used for the outcome of interest. The commercial source (Bioreliance) was reported
	Metric 15:	Number per Group	Medium	× 1	2	The number of strains tested (1) is lower than the typical number used in studies of a similar type (5). The number of replicates (n=3) for the single strain was appropriate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment methodology (revertant colony count) was described and appropriate for the outcome of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistency in outcome assessment between exposure groups and controls was inferred from the text.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable for the study design
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Not applicable for the study design
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Data were presented as means ± SEM of 3 replicates. Statistical analysis was performed using the Wilcoxon rank sum test.
	Metric 23:	Data Interpretation	Medium	× 2	4	Statistical significance was used to indicate a positive result. The criteria for the strength of mutagenicity were not reported. The study also indicates that "points where toxicity were observed were not included" [in determination of mutagenic activity]. It is not clear how this impacts the results
	Metric 24:	Cytotoxicity Data	Low	× 1	3	Specific assays for cytotoxicity were not included in the study design; however, the text indicated that toxicity was assessed based on microcolony formation or decreasing total number of revertants with increasing concentrations.
	Metric 25:	Reporting of Data	High	× 2	2	Data were reported graphically (mean and SE for 3 replicates); positive control data were not reported.

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Study Citation:	Irving, R.,Elfarra, A. A. (2013). Mutagenicity of the cysteine S-conjugate sulfoxides of trichloroethylene and tetrachloroethylene in the Ames test Toxicology, 306 157-161
Data Type:	Ames assay for PERC metabolites TCVC and TCVCS
HERO ID:	2128042

Domain	Metric	Rating†	MWF⋆	Score	Comments††
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 92: **In vitro evaluation results for Deferme et al 2015 for DNA strand break study**

Study Citation:	Deferme, L.,Wolters, J.,Claessen, S.,Briedé, J.,Kleinjans, J. (2015). Oxidative Stress Mechanisms Do Not Discriminate between Genotoxic and Nongenotoxic Liver Carcinogens Chemical Research in Toxicology, 28(8), 1636-1646
Data Type:	dsDNA breaks and 8-OHdG
HERO ID:	3489972

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	The test substance was identified by name as tetrachloroethylene (TCE).
	Metric 2:	Test Substance Source	High	× 1	1	The commercial source (Sigma-Aldrich) was reported.
	Metric 3:	Test Substance Purity	Low	× 1	3	Purity not reported
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent solvent (EtOH) controls were reported, but data was not shown.
	Metric 5:	Positive Controls	High	× 2	2	Positive controls (menadione, etoposide) were used when appropriate
	Metric 6:	Assay Procedures	Medium	× 1	2	Assays (gamma H2AX and 8-OHdG) were preformed as previously described or according to the manufacturer protocols. Brief details were provided.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	Not applicable for the study design
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	High	× 1	1	Limited details of test substance preparation (stock solution diluted into media to desired concentration at the time of the assay) were provided. Test substance storage was not provided, but this is appropriate given the study design (single-dose administration).
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Time matched controls were reported to be treated in an identical manner as the treatment group
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	The concentration used (2mM) was clearly stated
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	The exposure durations (24, 48, and 72hr) were clearly reported and appropriate for the outcomes of interest.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	The single exposure group was appropriate for the outcome of interest, however, the chosen concentration (reported to be the IC20 concentration based on previous MTT assays after 72hr exposure) was hypothesized to be the optimal dose for seeing gene expression changes which were evaluated in the same study. Since the DNA damage assay results were negative, it is unclear whether this concentration was truly appropriate for these specific outcomes.

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Study Citation: Deferme, L.,Wolters, J.,Claessen, S.,Briedé, J.,Kleinjans, J. (2015). Oxidative Stress Mechanisms Do Not Discriminate between Genotoxic and Nongenotoxic Liver Carcinogens Chemical Research in Toxicology, 28(8), 1636-1646
Data Type: dsDNA breaks and 8-OHdG
HERO ID: 3489972

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not included, but is not necessarily relevant to the outcome of interest.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The test model (Hep2 cells) was adequately described including passage number, commercial source, and detailed culture conditions/confluency prior to the test.
	Metric 15:	Number per Group	Medium	× 1	2	Three replicates were reported for each exposure duration. It was not specified if these were technical or biological replicates.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	Outcome assessment was adequately described and appropriate for the outcomes of interest.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were consistently assessed across study groups.
	Metric 18:	Sampling Adequacy	High	× 2	2	An appropriate number of cells (10,000/sample) were analyzed by flow cytometry.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	This metric is not applicable to the outcome of interest.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences between study group parameters. The same lot of cells were used for control and treatment groups.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	Confounding variables in outcomes unrelated to exposure were not reported.
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Appropriate statistical analysis (paired student's T-test) was used to determine differences between control and treatment groups.
	Metric 23:	Data Interpretation	High	× 2	2	Data interpretation was briefly described ("Cells with significant levels of g-H2Ax and 8-OHdG positive signals were presented as a percentage of total cells."); however, more details methods on gating procedures for analyzing flow cytometry results were not presented and may be presented in the cited references. However, the data interpretation appeared appropriate.

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Study Citation:	Deferme, L.,Wolters, J.,Claessen, S.,Briedé, J.,Kleinjans, J. (2015). Oxidative Stress Mechanisms Do Not Discriminate between Genotoxic and Nongenotoxic Liver Carcinogens Chemical Research in Toxicology, 28(8), 1636-1646
Data Type:	dsDNA breaks and 8-OHdG
HERO ID:	3489972

Domain	Metric	Rating†	MWF⋆	Score	Comments††
	Metric 24: Cytotoxicity Data	Medium	× 1	2	The concentration tested was previously determined to be the IC20. – Additional (concurrent) cytotoxicity assays were not performed/reported.
	Metric 25: Reporting of Data	High	× 2	2	Results for all samples/outcomes were adequately reported. Data was presented in figures (bar graphs) as means with SEM.
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

7 Developmental and Reproductive

Table 93: **Animal toxicity evaluation results of Carney et al 2006 for a gestational exposure study on reproductive, growth (early life) and development, nutrition and metabolic/adult exposure body weight, mortality outcomes**

Study Citation:	Carney, EW; Thorsrud, BA; Dugard, PH; Zablotny, CL (2006). Developmental toxicity studies in Crl:CD (SD) rats following inhalation exposure to trichloroethylene and perchloroethylene Birth Defects Research, Part B: Developmental and Reproductive Toxicology, 77(5), 405-412
Data Type:	Gestational exposure study - Perc
HERO ID:	630415

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	tetrachloroethylene (PERC)
	Metric 2:	Test Substance Source	Medium	× 1	2	INEOS CHlor Ltd, no batch number
	Metric 3:	Test Substance Purity	High	× 1	1	>99%
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	
	Metric 5:	Positive Controls	Not Rated	NA	NA	Not required by cited guidelines (OPPTS 870.370 and OECD 414)
	Metric 6:	Randomized Allocation	High	× 1	1	Animals were randomly assigned to four groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	Medium	× 1	2	The method and equipment used to generate the test substance as a gas, vapor, or aerosol were NOT reported. It is not clear if the vapor generation method reported for TCE was also used for PERC (different laboratories, different chambers, different flow rates, etc). However, since analytical concentrations were reported, omission of vapor generation details is unlikely to have a substantial impact on results
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	The concentrations of PERC were measured multiple times each exposure day using GC analysis. Exposure administration consistent across groups. (already downgraded metric 7 to unacceptable based on lack of methods for generating atmospheres, so that was not used to grade for this metric).
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and analytical exposure levels were reported..
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	GD 6-19, 6 hr/d, 7 d/wk; Both guidelines cited indicate that animals should be dosed until the day prior to C-section and sacrifice, which was reported as GD 20.

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Study Citation:	Carney, EW; Thorsrud, BA; Dugard, PH; Zablotny, CL (2006). Developmental toxicity studies in Crl:CD (SD) rats following inhalation exposure to trichloroethylene and perchloroethylene Birth Defects Research, Part B: Developmental and Reproductive Toxicology, 77(5), 405-412
Data Type:	Gestational exposure study - Perc
HERO ID:	630415

Domain	Metric		Rating†	MWF*	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	3 exposure and 1 control. These test concentrations were based on the results from the previously discussed developmental toxicity studies. The highest exposure level of 600ppm (equivalent to 4.1mg PERC/L) exceeds the limit concentration of 2 mg/L specified in the EPA prenatal developmental toxicity test guideline (OPPTS 870.3700).
	Metric 12:	Exposure Route and Method	High	× 1	1	Animals were whole body exposed in 0.75-cubic-meter exposure chambers. Chamber airflow was maintained at approximately 150 L/min. This resulted in approximately 12 air changes per hour.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Crl:CD (SD) rats (Charles River). Virgin female rats. Initial BW not reported (body weights reported fro GD 3, 6, 9, 13, 17, and 20).
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Housing adequately described. Room temperature and humidity were maintained within laboratory specific ranges (19–231C and 40–70% relative humidity). A 12-hr photoperiod was maintained for all animals. Food an water available ad libitum except during exposure periods.
	Metric 15:	Number per Group	High	× 1	1	22 dams/group; in accordance with guidelines
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Maternal toxicity - clinical signs, BW, feed consumption, mortality Reproductive/Devt - gravid uterine weights, placenta weight, # corpora lutea, uterine implants, resorptions, live/dead fetuses, fetal weight, external, skeletal, and visceral malformations/variations Although the current OECD test guideline 414 (updated in 2018) indicates that AGD should be measured in all live fetuses, the OECD TG 414 version available at the time of publication of this study was from 2001 and did not require measurement of AGD and the cited OPPTS guideline does not have that requirement.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation across groups
	Metric 18:	Sampling Adequacy	High	× 1	1	17-22 pregnant dams

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Study Citation:	Carney, EW; Thorsrud, BA; Dugard, PH; Zablotny, CL (2006). Developmental toxicity studies in Crl:CD (SD) rats following inhalation exposure to trichloroethylene and perchloroethylene Birth Defects Research, Part B: Developmental and Reproductive Toxicology, 77(5), 405-412
Data Type:	Gestational exposure study - Perc
HERO ID:	630415

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not done for PERC and not required by cited guidelines.
	Metric 20:	Negative Control Response	High	× 1	1	Control data reported. Historical control data discussed when needed to assess results.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Medium	× 2	4	Initial BW not reported; no statistically significant changes in BW during study. Only change in food consumption was 7% decrease in high-exposure group from GD 6-8. Respiratory rate not specifically mentioned, but no exposure-related clinical signs reported in dams, so bradyapnea unlikely. Downgraded to medium since PERC is a respiratory irritant (HSDB)
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	No mortalities, no clinical signs. Only attrition was time-mated females that were not pregnant (in all groups) that were not included in analysis.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Litter is statistical unit. Continuous data were tested in both studies for homogeneity of variance using Bartlett's test. The raw, log-transformed and square root–transformed data were tested. Based on results, data were analyzed using either parametric or nonparametric tests. If 75% of the data (across all groups) were the same value, then a frequency analysis was performed. Treatment groups were compared using a Mantel test for a trend in proportions and also pairwise Fisher's Exact tests were used for each dose group against the control. Skeletal variants were analyzed by a generalized mixed linear model with a logit link function and used litter as a random effect/ Each treated group was compared to the control group using a Wald chi-square test.
	Metric 24:	Reporting of Data	High	× 2	2	All reproductive and developmental findings were reported quantitatively in tabular or graphical format. maternal body weights and food consumption reported in tables. Mortality and clinical signs reported qualitatively (no exposure-related findings)
Overall Quality Determination‡			High		1.3	

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Study Citation:	Carney, EW; Thorsrud, BA; Dugard, PH; Zablotny, CL (2006). Developmental toxicity studies in Crl:CD (SD) rats following inhalation exposure to trichloroethylene and perchloroethylene Birth Defects Research, Part B: Developmental and Reproductive Toxicology, 77(5), 405-412
Data Type:	Gestational exposure study - Perc
HERO ID:	630415

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 94: **Animal toxicity evaluation results of Tinston et al 1994 for a multigeneration inhalation study on reproductive, growth (early life) and development, and renal outcomes**

Study Citation:	Tinston, DJ (1994). Perchloroethylene: A multigeneration inhalation study in the rat					
Data Type:	Multigeneration inhalation study					
HERO ID:	631041					
Domain		Metric	Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Idenitified by chemical name.
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer and lot no. were given.
	Metric 3:	Test Substance Purity	High	× 1	1	99.9% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	filtered air
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls are not used for multigeneration studies.
	Metric 6:	Randomized Allocation	High	× 1	1	The F0 parents were distributed amongst the four experimental groups after ensuring that any litters containing unhealthy individuals and litters at the extreme of the weight range were excluded from the randomization procedure. Allocation from within the litters was also at random. The F1, F1A and F2A litters and normal pups from each litter were randomly selected.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage were well described; analysis determined that stability was satisfactory.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Same exposure frequency, chamber design and animals per chamber.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	The authors report that the daily mean analyzed concentrations of Perchloroethylene were close to target.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	6h/day 5 day per week, except during mating and gestation (6h/day, 7 days/week)/
	Metric 11:	Number of Exposure Groups and Dose Spacing	Medium	× 1	2	3 exposure groups plus a control, not justified by study suthors, but dose response relationships were apparent.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Whole body chamber; unclear whether vapor would condense; 12 exchanges/hour were calculated from data provided.
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species and source were reported.

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Study Citation:	Tinston, DJ (1994). Perchloroethylene: A multigeneration inhalation study in the rat
Data Type:	Multigeneration inhalation study
HERO ID:	631041

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	All husbandry conditions were reported.
	Metric 15:	Number per Group	High	× 1	1	~25/sex/group
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	High	× 2	2	The outcome assessment methodology reported.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	
	Metric 18:	Sampling Adequacy	Medium	× 1	2	F2C litter inlcuded control and high dose group only.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not reported; however outcomes were objective.
	Metric 20:	Negative Control Response	Medium	× 1	2	Some clinical signs and histopath. lesions in controls.
Domain 6: Confounding / Variable Control						
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	Increased breathing rate was observed at 300 ppm; breathing irregulaties occurred at 1000 ppm;
	Metric 22:	Health Outcomes Unrelated to Exposure	Low	× 1	3	Problems with the lighting in the early part of the mating period; changes in pre-coital interval resulted from alterations in the photoperiod.
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 tables were provided for all outcomes.
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 95: **Animal toxicity evaluation results of Nelson et al 1979 for a neurodevelopmental inhalation study (gd 14-20) on growth (early life) and development and neurological/behavior outcomes**

Study Citation:	Nelson, BK; Taylor, BJ; Setzer, JV; Hornung, RW (1979). Behavioral teratology of perchloroethylene in rats Journal of Environmental Pathology, Toxicology and Oncology, 3(1-2), 233-250
Data Type:	Neurodevelopmental inhalation study (GD 14-20)
HERO ID:	58224

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Technical Grade-PCE;
	Metric 2:	Test Substance Source	Medium	× 1	2	TG-PERC obtained from Fisher Scientific; batch no. not reported, no independent analysis
	Metric 3:	Test Substance Purity	High	× 1	1	98.5% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	sham exposed group
	Metric 5:	Positive Controls	Not Rated	NA	NA	OECD guideline 426 (developmental neurotoxicity) states "To guard against possible false-negative findings and the inherent difficulties in “proving a negative,” available positive and historical control data should be discussed, especially when there are no treatment-related effects". However, positive control is not a requirement - especially since exposure-related effects were observed. Therefore, N/A was selected.
	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	Storage not reported. PERC was vaporized using heated flask, mixed with filtered room air and introduced into exposure chamber (airflow change rate 4x/min).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure conditions were identical for sham-exposed controls and exposure groups.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Only target exposure levels were reported. PERC levels in exposure chambers were continuously monitored by a Miran infrared analyzer and a charcoal tube sample was taken from the chamber air (generally one per day) and sent to an independent laboratory for gas chromatographic analysis. But results of analyses were not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	GD 14-20; 7 hr/d
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	2 exposure groups plus control; exposure levels selected based on dose-finding study

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Study Citation:	Nelson, BK; Taylor, BJ; Setzer, JV; Hornung, RW (1979). Behavioral teratology of perchloroethylene in rats Journal of Environmental Pathology, Toxicology and Oncology, 3(1-2), 233-250
Data Type:	Neurodevelopmental inhalation study (GD 14-20)
HERO ID:	58224

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Whole-body, dynamic chamber (0.41 cu m). Air flow 4 changes/min. Unclear how many animals per exposure chamber?
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Virgin male and female SD rats obtained from Harlan Industries and mated. Sperm-positive females used in study.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were consistent; pregnant females housed alone.
	Metric 15:	Number per Group	High	× 1	1	15-21 dams/group; litters culled to 4/sex within 16 hrs of delivery
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Comprehensive neurobehavioral testing, neurochemical analysis, and neurohistopathology was conducted on PND 4-46, using 1/sex per litter; pup body weights were also monitored. However, confidence downgraded to medium because maternal toxicity was not evaluated in this study (only pilot study).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation between groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	1/sex per litter in neurobehavioral testing (so litter is statistical unit)
	Metric 19:	Blinding of Assessors	Low	× 1	3	The study authors did not indicate whether or not assessors of neurobehavior were blinded. Certain tests contain subjective endpoints, which could have introduced bias. Pup body weight and histopathology do not require blinding.
	Metric 20:	Negative Control Response	Low	× 1	3	Control data reported. Study authors noted that offspring of animals sham-exposed from 7-13 (different study) and 14-20 (this study) differed. Study authors indicated that this stressed importance of appropriate controls; however, it could also indicate variation in control replicates.
Domain 6: Confounding / Variable Control						

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Study Citation:	Nelson, BK; Taylor, BJ; Setzer, JV; Hornung, RW (1979). Behavioral teratology of perchloroethylene in rats Journal of Environmental Pathology, Toxicology and Oncology, 3(1-2), 233-250
Data Type:	Neurodevelopmental inhalation study (GD 14-20)
HERO ID:	58224

Domain		Metric	Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	All females weighed 200-300 g at study initiation. Dam BW and food consumption were not reported for this study, but in the pilot study (which used high exposure level), no significant change in BW or food consumption was observed in exposed dams. Study authors did not indicate whether respiratory rate was measured. Since PERC is a respiratory irritant, confidence downgraded to low.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Multivariate ANOVA for most, open field and ascent tests analyzed with contingency tables; neurochemical data analyzed with 2-tailed students t-test
	Metric 24:	Reporting of Data	Medium	× 2	4	Control and high-exposure level data reported Graphical presentation of control and high-exposure level data was provided for some exposure-related endpoints; others were reported qualitatively as significant findings. Non-significant findings reported qualitatively. All low-exposure group data reported qualitatively (no exposure-related findings)
Overall Quality Determination‡			~~Medium~~ ⟶ Low§		~~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 =≥ 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: "Study was downgraded for the following reasons: 1) lack of blinding in neurobehavioral assessment (which was primary focus of study), 2) variation in control replicates, and 3) lack of evaluation of maternal effects in main study (only pilot study)."

Table 96: **Animal toxicity evaluation results of Halogenated Solvents, Indust for a multigen inhalation study in rats on reproductive, renal, hepatic, growth (early life) and development, neurological/behavior, nutrition and metabolic/adult exposure body weight outcomes**

Study Citation:	HSIA (Halogenated Solvents Industry Alliance) (1995). Perchloroethylene: Multigeneration inhalation study in the rat, with cover letter dated 07/06/95
Data Type:	Multigen inhalation study in rats
HERO ID:	4214380

Domain		Metric	Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by unambiguous name
	Metric 2:	Test Substance Source	High	× 1	1	Test substance source and lot number was identified and certificate of analysis provided.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity reported to be 99.9% (w/w).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Study does not explicitly state that controls were sham-treated, but descriptions of exposure imply sham-treatment: "the females in the control, 300, and 1000 ppm groups were exposed"
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not typical for this study type
	Metric 6:	Randomized Allocation	Medium	× 1	2	Study reports allocation method, which was semi random while preventing sibling matings.
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Preparation and storage conditions were reported, and stability was satisfactory. Methods for test atmosphere generation were reported and appropriate. Air changes per hour were appropriate (>10 based on chamber volume of 3400 L and air flow rate of 700 L/min).
	Metric 8:	Consistency of Exposure Administration	Medium	× 1	2	Animals were exposed in cages arranged vertically in the exposure chamber, which could allow for some inconsistencies in breathing zone concentrations if vertical mixing was inadequate (Perc is much more dense than air). In addition, the exposure frequency varied between 5 and 7 days per week at different phases of the study, but the frequencies were the same across different exposure groups.
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Analytical concentrations were reported and mean values were within 10% of nominal at all phases..
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure frequency and duration were typical for this study type

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Study Citation:	HSIA (Halogenated Solvents Industry Alliance) (1995). Perchloroethylene: Multigeneration inhalation study in the rat, with cover letter dated 07/06/95
Data Type:	Multigen inhalation study in rats
HERO ID:	4214380

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	High	× 1	1	Three nonzero exposure groups were used, with half log spacing. Exposure range was sufficient to enable identification of effect levels.
	Metric 12:	Exposure Route and Method	High	× 1	1	Inhalation study, adequately described
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Species, strain, sex, health status, age, body weight, and source were reported and appropriate.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	Low	× 1	3	Authors noted that faulty light switches altered the light cycle for F0 parents and this alteration may have been responsible for reduced pre-coital interval in exposed groups.
	Metric 15:	Number per Group	High	× 1	1	All groups consisted of 24/sex. EPA guidelines call for group size yielding 20 pregnant females so group size was appropriate.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Outcome assessment methodology was reported. Neither sperm parameters nor estrus cyclicity was evaluated; water intake was not measured. In addition, only testes, kidneys, and liver weights were obtained (EPA guidelines recommend several other organ weights), and histopathology did not include organs typically assessed in this study type (e.g., pituitary and adrenal glands). Ages at vaginal opening/preputial separation were not evaluated in F1 offspring.
	Metric 17:	Consistency of Outcome Assessment	Medium	× 1	2	Histopathology examinations were not consistent across animals. Histopathology examinations were initially limited to liver and kidney of control and high dose animals, and reproductive organs of suspected infertile animals. Additional groups were evaluated for liver and kidney histopathology but the assessment was not consistent across groups. HIstologic examination of testes was extended to fertile F1 males, necessitating re-examination of infertile males for consistency.
	Metric 18:	Sampling Adequacy	High	× 1	1	Sampling was reported and appropriate; endpoints evaluated in all exposed animals.
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Study did not report blinding for clinical observations, but the main outcomes assessed were not subjective.

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Study Citation:	HSIA (Halogenated Solvents Industry Alliance) (1995). Perchloroethylene: Multigeneration inhalation study in the rat, with cover letter dated 07/06/95
Data Type:	Multigen inhalation study in rats
HERO ID:	4214380

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	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	No confounding factors apart from the lighting malfunction in the first generation were noted. Respiratory rate was not reported.
	Metric 22:	Health Outcomes Unrelated to Exposure	High	× 1	1	Authors noted that there was no evidence of disease or infection that might have affected outcomes.
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Statistical analysis was performed, described, and appropriate to the outcomes.
	Metric 24:	Reporting of Data	High	× 2	2	All data were presented graphically or in tabular form, with measures of variability.
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: "Study was generally well conducted but evaluations were limited and performed inconsistently"

Table 97: **Animal toxicity evaluation results of Nelson et al 1979 for a neurodevelopmental inhalation study (gd 7-13) study on growth (early life) and development outcomes**

Study Citation:	Nelson, BK; Taylor, BJ; Setzer, JV; Hornung, RW (1979). Behavioral teratology of perchloroethylene in rats Journal of Environmental Pathology, Toxicology and Oncology, 3(1-2), 233-250
Data Type:	Neurodevelopmental inhalation study (GD 7-13)
HERO ID:	58224

Domain	Metric		Rating[†]	MWF[⋆]	Score	Comments[††]
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	TG-PERC
	Metric 2:	Test Substance Source	Medium	× 1	2	TG-PERC obtained from Fisher Scientific; batch no. not reported, no independent analysis
	Metric 3:	Test Substance Purity	High	× 1	1	98.5% pure
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	sham exposed group
	Metric 5:	Positive Controls	Not Rated	NA	NA	OECD guideline 426 (developmental neurotoxicity) states "To guard against possible false-negative findings and the inherent difficulties in “proving a negative,” available positive and historical control data should be discussed, especially when there are no treatment-related effects". However, positive control is not a requirement - especially since exposure-related effects were observed. Therefore, N/A was selected.
	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	Storage not reported. PERC was vaporized using heated flask, mixed with filtered room air and introduced into exposure chamber (airflow change rate 4x/min).
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Exposure conditions were identical for sham-exposed controls and exposure group.
	Metric 9:	Reporting of Doses/Concentrations	Low	× 2	6	Only target exposure levels were reported. PERC levels in exposure chambers were continuously monitored by a Miran infrared analyzer and a charcoal tube sample was taken from the chamber air (generally one per day) and sent to an independent laboratory for gas chromatographic analysis. But results of analyses were not reported.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	GD 7-13; 7 hr/d

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Study Citation:	Nelson, BK; Taylor, BJ; Setzer, JV; Hornung, RW (1979). Behavioral teratology of perchloroethylene in rats Journal of Environmental Pathology, Toxicology and Oncology, 3(1-2), 233-250
Data Type:	Neurodevelopmental inhalation study (GD 7-13)
HERO ID:	58224

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only exposure group plus control (unacceptable based on PECO statement), but the use of multiple exposure levels within the exposed group (GD 7-13, GD 14-20) mitigates this concern; exposure level selected based on dose-finding study.
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Whole-body, dynamic chamber (0.41 cu m). Air flow 4 changes/min. Unclear how many animals per exposure chamber?
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	High	× 2	2	Virgin male and female SD rats obtained from Harlan Industries and mated. Sperm-positive females used in study.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	Husbandry conditions were consistent; pregnant females housed alone.
	Metric 15:	Number per Group	High	× 1	1	13-19 dams/group; litters culled to 4/sex within 16 hrs of delivery
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	Comprehensive neurobehavioral testing, neurochemical analysis, and neurohistopathology was conducted on PND 4-46, using 1/sex per litter; pup body weights were also monitored. However, confidence downgraded to medium because maternal toxicity was not evaluated in this study (only pilot study).
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Consistent evaluation between groups.
	Metric 18:	Sampling Adequacy	High	× 1	1	1/sex per litter in neurobehavioral testing (so litter is statistical unit)
	Metric 19:	Blinding of Assessors	Low	× 1	3	The study authors did not indicate whether or not assessors of neurobehavior were blinded. Certain tests contain subjective endpoints, which could have introduced bias. Pup body weight and histopathology do not require blinding.
	Metric 20:	Negative Control Response	Low	× 1	3	Control data reported. Study authors noted that offspring of animals sham-exposed from 7-13 (this study) and 14-20 (additional study) differed. Study authors indicated that this stressed importance of appropriate controls; however, it could also indicate variation in control replicates.
Domain 6: Confounding / Variable Control						

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Study Citation:	Nelson, BK; Taylor, BJ; Setzer, JV; Hornung, RW (1979). Behavioral teratology of perchloroethylene in rats Journal of Environmental Pathology, Toxicology and Oncology, 3(1-2), 233-250
Data Type:	Neurodevelopmental inhalation study (GD 7-13)
HERO ID:	58224

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 21:	Confounding Variables in Test Design and Procedures	Low	× 2	6	All females weighed 200-300 g at study initiation. Dam BW and food consumption were not reported for this study, but in the pilot study (which used the same exposure level), no significant change in BW or food consumption was observed in exposed dams. Study authors did not indicate whether respiratory rate was measured. Since PERC is a respiratory irritant, confidence downgraded to low.
	Metric 22:	Health Outcomes Unrelated to Exposure	Medium	× 1	2	data on attrition and/or health outcomes unrelated to exposure for each study group were not reported because only substantial differences among groups were noted
Domain 7: Data Presentation and Analysis						
	Metric 23:	Statistical Methods	High	× 1	1	Multivariate ANOVA for most, open field and ascent tests analyzed with contingency tables; neurochemical data analyzed with 2-tailed students t-test
	Metric 24:	Reporting of Data	Medium	× 2	4	Graphical presentation of control and exposure group data was provided for some exposure-related endpoints; others were reported qualitatively as significant findings. Non-significant findings reported qualitatively.
Overall Quality Determination‡			~~Medium~~ ⟶ Low§		~~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

§ Evaluator's explanation for rating change: "Study was downgraded for the following reasons: 1) lack of blinding in neurobehavioral assessment (which was primary focus of study), 2) variation in control replicates, 3) lack of evaluation of maternal effects in main study (only pilot study), and 4) only one exposure level"

Table 98: **Animal toxicity evaluation results of Beliles et al 1980 for a gestational exposure inhalation study on growth (early life) and development outcomes**

Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	Gestational exposure inhalation
HERO ID:	58331

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Identified by chemical name and synonym
	Metric 2:	Test Substance Source	High	× 1	1	Manufacturer and lot number given.
	Metric 3:	Test Substance Purity	Medium	× 1	2	91% pure, impurities were not characterized (PCE), 99.9% pure for TCE
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	High	× 2	2	Filtered air controls; control animals exposed in a different room.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls not used in developmental studies.
	Metric 6:	Randomized Allocation	High	× 1	1	randomly assigned to groups
Domain 3: Exposure Characterization						
	Metric 7:	Preparation and Storage of Test Substance	High	× 1	1	Method and equipment used to generate the test substance as a vapor were reported and appropriate.
	Metric 8:	Consistency of Exposure Administration	High	× 1	1	Chambers at 500ppm showed less than 2.5% variation throughout
	Metric 9:	Reporting of Doses/Concentrations	High	× 2	2	Target and analytical concentrations were provided. Range of measure concentration did not deviate more than 10%.
	Metric 10:	Exposure Frequency and Duration	High	× 1	1	Exposure throughout gestation or GD 6-18; 7 hours/day.
	Metric 11:	Number of Exposure Groups and Dose Spacing	Low	× 1	3	Only 1 exposure concentration was used (500ppm). .
	Metric 12:	Exposure Route and Method	Medium	× 1	2	Dynamic chamber , whole body, it is assumed that the substance does not condense. Number of air changes not indicated
Domain 4: Test Organism						
	Metric 13:	Test Animal Characteristics	Medium	× 2	4	Species, strain and source were reported; starting age and bw not given.
	Metric 14:	Adequacy and Consistency of Animal Husbandry Conditions	High	× 1	1	well reported
	Metric 15:	Number per Group	High	× 1	1	~20/group

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Study Citation:	Beliles, RP; Brusick, DJ; Mecler, FJ (1980). Teratogenic-mutagenic risk of workplace contaminants: trichloroethylene, perchloroethylene, and carbon disulfide
Data Type:	Gestational exposure inhalation
HERO ID:	58331

Domain	Metric	Rating†	MWF⋆	Score	Comments††
Domain 5: Outcome Assessment					
	Metric 16: Outcome Assessment Methodology	High	× 2	2	
	Metric 17: Consistency of Outcome Assessment	High	× 1	1	
	Metric 18: Sampling Adequacy	High	× 1	1	Litter data provided for applicable outcome
	Metric 19: Blinding of Assessors	Medium	× 1	2	Blinding was not reported, but most outcomes were not subjective.
	Metric 20: Negative Control Response	Low	× 1	3	Visceral and skeletal effects seen in controls
Domain 6: Confounding / Variable Control					
	Metric 21: Confounding Variables in Test Design and Procedures	Low	× 2	6	Respiratory rate was not measured; the chemical is a respiratory irritant.
	Metric 22: Health Outcomes Unrelated to Exposure	Medium	× 1	2	subcutaneous hematomas observed in all groups, including controls
Domain 7: Data Presentation and Analysis					
	Metric 23: Statistical Methods	High	× 1	1	Statistics were well described and appropriate
	Metric 24: Reporting of Data	High	× 2	2	All outcome were reported.
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

8 Mechanistic

Table 99: **In vitro evaluation results of Seo et al 2012 for mechanistic-allergic response study**

Study Citation:	Seo, M., Kobayashi, R., Okamura, T., Ikeda, K., Satoh, M., Inagaki, N., Nagai, H., Nagase, H (2012). Enhancing effects of trichloroethylene and tetrachloroethylene on type I allergic responses in mice Journal of Toxicological Sciences, 37(2), 439-445
Data Type:	Mechanistic-allergic response
HERO ID:	2128339

Domain	Metric		Rating†	MWF⋆	Score	Comments††
Domain 1: Test Substance						
	Metric 1:	Test Substance Identity	High	× 2	2	Test substance identified by name as trichloroethylene
	Metric 2:	Test Substance Source	High	× 1	1	The source (Nacalai Tesque Co Ltd.) was identified.
	Metric 3:	Test Substance Purity	High	× 1	1	Test substance purity was provided (98%).
Domain 2: Test Design						
	Metric 4:	Negative and Vehicle Controls	Medium	× 2	4	Concurrent negative controls were used. Authors did not specify whether untreated or vehicle controls were used but noted that the solvent (DMSO) did not affect experiments.
	Metric 5:	Positive Controls	Not Rated	NA	NA	Positive controls were not required.
	Metric 6:	Assay Procedures	High	× 1	1	Assay procedures were described and applicable for the study type.
	Metric 7:	Standards for Tests	Not Rated	NA	NA	No standards were required.
Domain 3: Exposure Characterization						
	Metric 8:	Preparation and Storage of Test Substance	Low	× 1	3	Preparation was reported, but no information on methods used to prevent volatilization during preparation was reported. Storage information was not reported.
	Metric 9:	Consistency of Exposure Administration	High	× 1	1	Details of exposure administration were reported and consistent across groups.
	Metric 10:	Reporting of Doses/Concentrations	High	× 2	2	Concentrations were reported in mg/L
	Metric 11:	Number of Exposure Groups and Concentration Spacing	High	× 2	2	Duration of exposure (30 min) was reported.
	Metric 12:	Exposure Route and Method	High	× 1	1	The number of exposure groups (3 plus control) was reported and concentrations justified (values similar to Japanese standard for drinking water). Tested concentrations yielded a range of responses.
	Metric 13:	Metabolic Activation	Not Rated	NA	NA	Metabolic activation was not required.
Domain 4: Test Model						
	Metric 14:	Test Model	High	× 2	2	The source, cell type, and culturing methods were reported.

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Study Citation:	Seo, M., Kobayashi, R., Okamura, T., Ikeda, K., Satoh, M., Inagaki, N., Nagai, H., Nagase, H (2012). Enhancing effects of trichloroethylene and tetrachloroethylene on type I allergic responses in mice Journal of Toxicological Sciences, 37(2), 439-445
Data Type:	Mechanistic-allergic response
HERO ID:	2128339

Domain	Metric		Rating†	MWF⋆	Score	Comments††
	Metric 15:	Number per Group	High	× 1	1	The number of cells used and number of experiments (3 replicates) were reported.
Domain 5: Outcome Assessment						
	Metric 16:	Outcome Assessment Methodology	Medium	× 2	4	The method for determining histamine release was partially reported and cited to another publication.
	Metric 17:	Consistency of Outcome Assessment	High	× 1	1	Outcomes were assessed consistently.
	Metric 18:	Sampling Adequacy	Not Rated	NA	NA	Not applicable to outcome
	Metric 19:	Blinding of Assessors	Not Rated	NA	NA	Blinding not required for outcomes.
Domain 6: Confounding / Variable Control						
	Metric 20:	Confounding Variables in Test Design and Procedures	High	× 2	2	There were no differences reported among study group parameters that could influence the outcome assessment.
	Metric 21:	Confounding Variables in Outcomes Unrelated to Exposure	Medium	× 1	2	data on experienced disproportionate outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis						
	Metric 22:	Data Analysis	High	× 1	1	Statistical methods were described and data fully reported graphically.
	Metric 23:	Data Interpretation	Not Rated	NA	NA	Criteria not required.
	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 were reported graphically for all treatment groups (mean, SE, and number replicates) for the outcome of interest.
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 $=\geq 1$ 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